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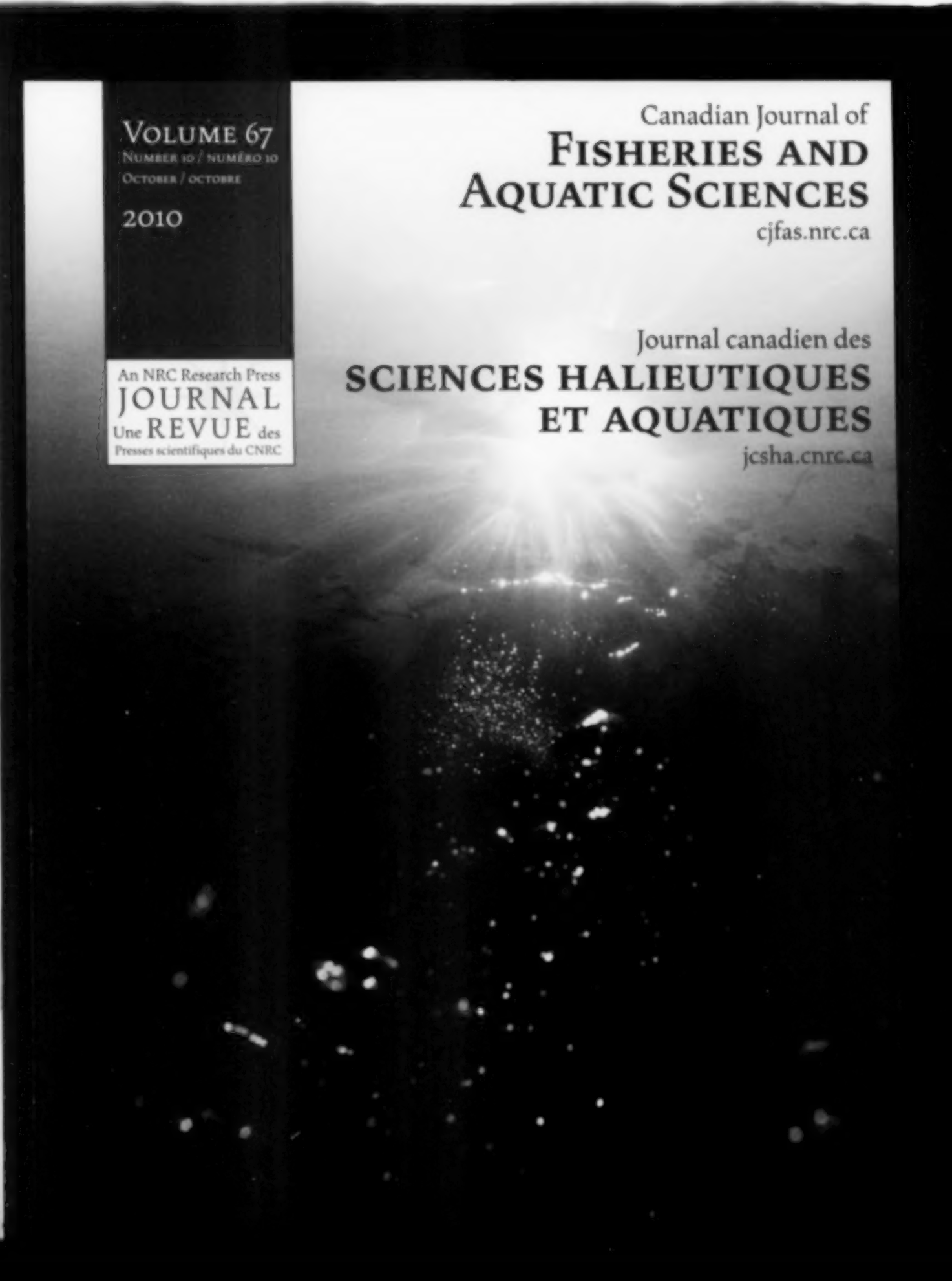

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Integrated stock mixture analysis for continous and categorical data, with application to genetic–otolith combinations

Stephen J. Smith and Steven E. Campana

Abstract: Fish populations or stocks often intermix on fishing grounds, thus posing problems for stock assessors or managers attempting to optimize yields and minimize overexploitation of individual stocks. A Bayesian framework is developed here to simultaneously analyse many of the different data types (e.g., otolith elemental composition, nuclear and mitochondrial DNA) that have been used to identify stock origins of fish in mixed groups and thus take maximal advantage of the available information. Elements of this framework include the capability to analyse each data type either separately or in combination for any number of mixed-group samples, Bayesian credible intervals to evaluate the uncertainty associated with the estimated proportion of the original stocks in the mixed groups, and posterior predictive diagnostics to evaluate the assumptions of the underlying models. The framework was used to re-analyse a subset of otolith elemental composition and microsatellite allele frequency data obtained from the same fish from Atlantic cod (*Gadus morhua*) stocks in the Gulf of St. Lawrence, Canada.

Résumé : Les populations ou les stocks de poissons se mélangent souvent sur les aires de pêche, ce qui présente des problèmes pour les évaluateurs de stocks et les gestionnaires qui essaient d'optimiser les rendements et minimiser la surexploitation des stocks individuels. Nous mettons au point ici un cadre bayésien pour analyser simultanément plusieurs des différents types de données (par ex., composition des otolithes en éléments, ADN nucléaire et mitochondrial) qui servent à identifier l'origine des stocks de poissons en peuplements mixtes, pour ainsi tirer un avantage maximal des renseignements disponibles. Ce cadre comporte plusieurs éléments, dont la capacité d'analyser chaque type de données ou bien séparément ou alors en combinaison pour tout nombre d'échantillons de peuplements mixtes, des intervalles bayésiens crédibles pour évaluer l'incertitude associée à la proportion estimée des stocks originaux dans les peuplements mixtes, ainsi que des diagnostics prédictifs a posteriori pour évaluer les présuppositions des modèles sous-jacents. Nous utilisons le cadre pour analyser de nouveau un sous-ensemble de données de compositions des otolithes en éléments et de fréquences alléliques des microsatellites des mêmes poissons provenant de stocks de morues franches (*Gadus morhua*) du golfe du Saint-Laurent, Canada.

[Traduit par la Rédaction]

Introduction

Trace elemental composition of otoliths is frequently used as a natural tag to identify and track fish aggregations or populations as they move and has been used for identifying and classifying fish in a mixture from various sources (Elsdon et al. 2008) in much the same way as genetic markers are used (Waples et al. 2008). In a number of cases, other material often assumed to be population-specific such as DNA, meristics, and morphometrics are also collected on these populations (e.g., Milton and Chenery 2001; Berg et al. 2005; Miller et al. 2005) and analyzed separately from the otolith data. These different analyses can result in the same fish in the mixed samples being assigned to different source populations depending on the data type. Reconciling the differences in assignment can be difficult when different analytical methods are used and the various kinds of data are analyzed separately. Ideally, one would want to analyse all of the different data types in the same analysis and thus take maximal advantage of the available information.

One of the more common methods for analyzing stock composition data was developed in a maximum likelihood context by Fournier et al. (1984) and then modified by Millar (1987) to use the E-M algorithm. This method begins with a set of known source or base populations for which observations of otolith data, genetic data, or other population-specific measurements are available. A specimen from the mixed samples of unknown origin is assigned to the base population for which the likelihood of their observations and population parameters is a maximum. Pella and Masuda (2001) introduced a Bayesian form of the method

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with specimens from the mixed samples being assigned to base populations based on maximum posterior probabilities.

Classification of a mixture can be based on conditional or unconditional estimation whether one uses a maximum likelihood or a Bayesian approach (Pella and Masuda 2005). In the conditional case, only the parameter estimates from the base populations are used to classify the mixed samples. Examples of this approach are Fournier et al. (1984) and Millar (1987) for maximum likelihood estimation and Munch and Clarke (2008) for a Bayesian formulation. For unconditional estimation, the observations from the mixed samples classified to a base population are used to update the parameter estimates of the base population (e.g., Smouse et al. (1990) for an early maximum likelihood approach and Pella and Masuda (2001) for a Bayesian approach).

The classification model was originally defined to work with continuous random variables such as otolith elemental composition or morphometric measurements, discrete random variables such as frequencies of alleles or meristic measurements, or both continuous and discrete random variables at the same time (Fournier et al. 1984; Millar 1987). However, an integrated analysis has never been reported, in large part due to the absence of an analytical framework. Programs such as HISEA (conditional maximum likelihood; Millar 1990) and Matlab code for conditional Bayesian estimation available from Munch and Clarke (2008) are available for continuous random variables. There are more programs available that are only designed for discrete or genetic data: SPAM (conditional maximum likelihood; Debevec et al. 2000), BAYES (unconditional Bayesian estimation; Pella and Masuda 2001), cBAYES (C++ version of BAYES; Beacham et al. 2005), ONCOR (conditional maximum likelihood; Kalinowski et al. 2007), and Mixstock (unconditional Bayesian estimation in an R package with WinBugs for haplotype data; Bolker 2007). Users can write their own subroutines in FORTRAN to evaluate the likelihood of discrete random variables in HISEA.

At present, the separate and joint analyses of otolith, genetic, or other kinds of data for determining stock composition do not have a coherent and accessible framework for estimation, evaluation, and diagnostics. Although all of the components to do this have been developed, they have never been integrated into a common approach. The objective of this paper is to unify the analysis of these data in a Bayesian framework, thus allowing unknown mixtures to be classified using either otolith elemental or genetic data or both sources combined. Our method is not restricted to these two kinds of data, and the same general Bayesian model can be used with likelihoods specific to the data type. The approach developed herein was applied to a subset of genetic and otolith data collected on Atlantic cod (*Gadus morhua*) from the Gulf of St. Lawrence in 1996 and 1997. These data were originally analysed for stock composition with Campana et al. (1999) using the otolith elemental data and Ruzzante et al. (2000) using the microsatellite allele frequency data. Both of these studies used the conditional maximum likelihood version of the stock composition model. A second objective of the paper is to assess the additional information content available to researchers when multiple data types are analyzed in a single framework.

Materials and methods

Sample collection and data preparation

To characterize the genetic and otolith elemental fingerprints of each of the major cod stocks in and around the Gulf of St. Lawrence, samples of adult cod (35–85 cm) in spawning or near-spawning condition were collected in spring 1996 and 1997 from each of the four known spawning grounds: (1) the southern Gulf of St. Lawrence (NAFO Division 4T), in both the southwest (Shediac Valley) and southeast (off Cheticamp); (2) the northern Gulf of St. Lawrence (NAFO Division 3Pn4RS), primarily near the mouth of St. George's Bay; (3) southern Newfoundland (NF) (NAFO Division 3Ps), both offshore on St. Pierre Bank (in 1996) and inshore in Fortune and Placentia bays (in 1997); and (4) the Scotian Shelf (NAFO Division 4Vs), to both the east (the Gully) and the north (Banquereau Bank) (Table 1; Fig. 1). Each spawning ground was sampled at least twice in any given year, and most spawning grounds were sampled in both 1996 and 1997 (Fig. 1). A minimum of 100 cod was generally collected from each stock, for a total of 1111 fish. The sagittal otolith pair was removed from each fish immediately after capture, as well as a 1 mL sample of blood or a sample of soft muscle tissue immediately posterior to the tongue. Blood and muscle samples were immediately preserved in 95% ethanol, whereas otoliths were stored dry. These samples comprised our base populations, as the stock identity of each of these fish was known with some confidence. Only fish for which matching otolith and genetic assays were available were analyzed in the current analysis.

Several cod stocks are known to overwinter in large aggregations near the mouth of the Gulf of St. Lawrence (Campana et al. 1999). A winter fishery on these mixtures thus poses serious problems for stock assessment and management. A grid sampling design was used to collect the mixed samples of cod in and around the approaches to the Gulf of St. Lawrence each January in 1996 and 1997. Bottom trawl surveys were carried out on 3–22 January 1996 on the CCGS *Wilfred Templeman* (WT182) and on 6–25 January 1997 on the CCGS *Wilfred Templeman* (WT201). The nominal distance between survey samples was 20 km, although some areas were surveyed at 5 km intervals. Otoliths for determination of stock identity were collected from adult cod (35–85 cm) on approximately every second set (Table 1; Fig. 1). A total of 138 successful sets were made in 1996, of which 60 sets were sampled for otoliths (n = 754). Samples from 1997 came from 104 successful sets, of which 49 sets were sampled for otoliths (n = 866). Additional samples (n = 89; 45°50′N latitude, 58°03′W longitude) to the east of the region surveyed by the CCGS *Wilfred Templeman* in 1997 were collected on 15 March 1997 in two sets as part of the CCGS *Alfred Needler* N255 spring survey. Sample processing was as described for the summer samples and once again was restricted to fish for which both genetic and otolith information were available.

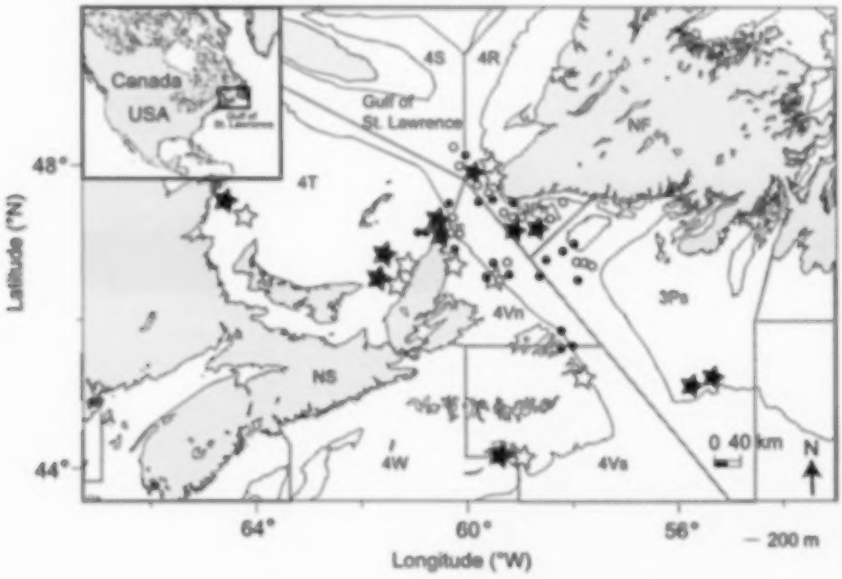
Each of the cod populations off the eastern coast of Canada spawns at a distinct site and thus is of known population identity at the time of spawning (Templeman 1962). Because the otolith elemental fingerprints of the 1996 and 1997 spring spawning aggregations differed significantly among spawning groups (Campana et al. 1999), they were

Table 1. Details on data used in the classification analysis.

		Sample size		No. of alleles					
Year	Location	One	Both	Gmo2	Gmo4	Gmo120	Gmo132	Gmo145	Gmo151
Base samples									
1996	3Pn4RS	179	130	17	40	32	11	34	43
	3Ps	93	52	13	28	28	7	25	31
	4T	200	179	17	33	34	11	44	45
	4Vs	100	93	15	32	27	16	37	37
	All areas	572	454	22	47	40	19	54	61
1997	3Pn4RS	134	47	10	28	24	8	24	28
	4T	200	64	14	28	29	5	30	38
	4Vn	48	39	11	26	22	7	22	15
	4Vs	150	82	13	31	31	8	28	33
	All areas	532	222	17	40	139	9	40	47
Mixed samples									
1996	3Pn4RS	198	74	13	28	28	10	31	40
	3Ps	169	61	13	33	26	9	32	36
	4Vn	324	126	13	32	32	9	33	41
	4Vs	49	39	10	23	24	8	27	25
	All areas	740	320	16	41	36	11	49	57
1997	3Pn4RS	528	254	16	42	36	9	40	49
	3Ps	255	88	12	31	34	6	28	38
	4T	77	28	11	24	20	8	25	27
	4Vn	152	90	13	29	29	7	33	38
	All areas	1012	460	20	46	38	10	47	59

Note: Location refers to NAFO subarea (see Fig. 1). Sample size in the column "One" indicates number of observations when either otolith elemental composition or allele frequencies were measured. The number of observations with both measurements are given in column "Both".

Fig. 1. Map of study area in eastern Canada. Sample locations for spring spawners (stars) and winter mixed samples (circles) are indicated for 1996 (solid symbols) and 1997 (open symbols). Province of Nova Scotia (NS) and the island of Newfoundland (NF) are labelled on the map.



used as population- and year-specific reference markers against which the winter samples could be compared (Table 1). The elemental fingerprint of each otolith was characterized by a suite of five elements (Ba, Li, Mg, Mn, Sr) using isotope dilution inductively coupled plasma mass spectrometry and procedures described in Campana et al. (1999, 2000). Elemental assays for both spring spawner (known identity, base samples) and winter (unknown iden-

tity, mixed samples) samples from each year were systematically randomized with respect to assay sequence and analyzed together at the same laboratory. As a result, spring spawner and winter samples from any given year are completely comparable. As in previous analysis, the natural log transformation of the observations for the elements of Li and Mn was used to meet the normal distribution assumption for the classification model. Three elements (Mg, Mn, and Sr) were corrected for fish length. Full methodological details, including data transformations and calibration adjustments for fish size, are presented in Campana et al. (1999, 2000).

Nuclear DNA was extracted from the blood of many of the same cod as were analyzed by Campana et al. (1999, 2000), although the total sample size for the DNA analysis was smaller (see Table 1). DNA was analyzed as described in Ruzzante et al. (2000). Briefly, polymerase chain reaction (PCR) was used to amplify six microsatellite loci: Gmo2, Gmo132, Gmo145 (Brooker et al. 1994), Gmo4 (Wright 1993), Gmo120 (Ruzzante et al. 1996), and Gmo151 (Ruzzante et al. 2000). The resulting products were size-fractionated using 6.5% denaturing polyacrylamide gels, and alleles were sized by comparison with M13mp18 DNA sequencing reaction products.

Estimates and tests of genetic differentation (F_{ST}) were obtained using the computer package described in Goudet (2001).

Mixture model

The likelihood for the observations from the base tows can be written as

$$(1) \qquad l(\boldsymbol{\theta}) = \prod_{b=1}^{B}\prod_{i=1}^{n_b} f_b(x_{b,i};\boldsymbol{\theta})$$

for observation i from base population b. For the otolith elemental composition data, the distribution function $f_b(x_{b,i};\boldsymbol{\theta})$ was a multivariate normal for the otolith elemental compositional data with parameter set $\boldsymbol{\theta} = (\boldsymbol{\mu}_b, \boldsymbol{\Sigma})$. The distribution function was a multinomial distribution for the allele frequency data with parameter set $\boldsymbol{\theta}$ equal to $(N_{jb}, \boldsymbol{p}_{jb})$, where $\boldsymbol{p}_{jb} = (p_1, p_2, \ldots, p_K)_{jb}$ represents the probability of observing the kth allele ($k = 1, \ldots, K$) at locus j in the bth base population.

The likelihood for the mixture observations over A areas (winter samples), x'_{ami}, is similarly (for example, see Ripley 1996),

$$(2) \qquad l'(\boldsymbol{\theta}) = \prod_{a=1}^{A}\prod_{m=1}^{M}\prod_{i=1}^{n_{am}}\sum_{b=1}^{B} w_{amb} f_b(x'_{ami};\boldsymbol{\theta})$$

where w_{amb} represents the mixing or the stock composition proportions to be estimated for observations from area a, material m (otoliths or alleles), and base population b. Published applications of stock composition analysis generally concentrate on analysing one mixed sample; however, in our experience, observations are available from a number of sites for which stock compositions may differ. Given that the observations from the mixed samples, once assigned to respective base populations, will be used to estimate the parameters for those base populations, it makes sense to analyse all of the mixed samples at once (see discussion in Bolker et al. 2007).

Note that the two likelihoods given above share the same parameter set $\boldsymbol{\theta}$, implying that parameter estimation will be unconditional. For likelihoods of the joint distribution for the otolith and allele frequency data, $g_b(x;(\boldsymbol{\mu}_b, \boldsymbol{\Sigma})) \times h_b(x;(N_{jb}, \boldsymbol{p}_{jb}))$ would be substituted for $f_b(x;\boldsymbol{\theta})$ in eqs. 1 and 2 above.

The Bayesian model uses the combination of the likelihoods in eqs. 1 and 2 with prior distributions set for w_{amb} and the elements of $\boldsymbol{\theta}$. A Dirichlet distribution, $D(\boldsymbol{\alpha})$, was used as a prior for w_{amb} with $\alpha_b = 1/B \forall b$. Pella and Masuda (2001) recommend setting α_b to $1/B$ for all i so that the mean, variances, and covariances of the resulting posterior distributions reflect the combined increase in the sizes of the base and mixed samples. Noninformative normal priors were used for the otolith element means, with means 0 and variances 10^6. The common variance–covariance matrix was modelled using a Wishart distribution with the parameter set to the rank of the matrix (here equal to five for the five elements used).

Noninformative priors for the $\boldsymbol{p}_{jb}$ can be set using 1 over the number alleles at locus j in base population b as recommended by Rannala and Mountain (1997). However, Pella and Masuda (2001) argue that the prior means should reflect the similarities between the allele frequencies among the base samples and the prior as a whole should allow for a realistic assessment of the uncertainty in the genetic composition of the stock. They developed a pseudo-Bayes estimate for an informative prior based on differences between the allele frequencies for each base population and the mean over all base samples. For those loci with large differences from the mean, the prior will result in the posterior being closer to the observed frequencies. For those loci that are very similar to the mean over all base samples, the posterior will be shrunk towards the prior mean.

Symmetric credible intervals based on the posterior distribution were calculated for the proportion assigned to each of the base groups. Overlap of these intervals was interpreted as indicating equivocal assignment to the base groups.

The classification models were written in WinBUGS (Lunn et al. 2000). The pseudo-Bayes method used here was based on R code from Bolker (2007). Hardy–Weinberg equilibrium was assumed for the allele frequency data. For each model, a total of 10 000 Markov chain Monte Carlo (MCMC) samples (Gibbs sampler) for each of two chains were taken from the posterior distribution, with the first 5000 discarded for burn-in. Convergence to the posterior was evaluated using the Brooks–Gelman test (Brooks and Gelman 1998). An R package (R Development Core Team 2007) for analyzing these kinds of data using the methods presented in this paper is available from the authors.

A natural check on the classification model is to determine if the data observed from the base populations are still plausible under the posterior distribution (Gelman et al. 2004) once the mixed-sample observations have been classified. That is, we can use the posterior distribution to predict the observations that we would expect to see if the data collection was replicated and compare these with those that we had observed in first place.

The fit of the model was evaluated for the otolith data by

Table 2. Proportion of each 1996 mixed sample assigned to each of the 1996 base samples using either otolith elemental composition or allelic frequency data or both kinds of data combined.

			Base sample			
Mixed sample	Material	Prior	3Pn4RS	3Ps	4T	4Vs
3Pn4RS	Otoliths		0.879	0.010	0.019	0.093
	Alleles	Pseudo-Bayes	0.336	0.179	0.463	0.023
		Equal proportions	0.377	0.112	0.491	0.019
	Combined	Pseudo-Bayes	0.840	0.012	0.134	0.013
		Equal proportions	0.780	0.013	0.200	0.006
3Ps	Otoliths		0.718	0.248	0.014	0.020
	Alleles	Pseudo-Bayes	0.169	0.175	0.613	0.044
		Equal proportions	0.241	0.095	0.622	0.042
	Combined	Pseudo-Bayes	0.554	0.211	0.220	0.015
		Equal proportions	0.572	0.132	0.280	0.017
4Vn	Otoliths		0.038	0.008	0.939	0.015
	Alleles	Pseudo-Bayes	0.371	0.023	0.578	0.029
		Equal proportions	0.351	0.016	0.610	0.024
	Combined	Pseudo-Bayes	0.195	0.006	0.792	0.007
		Equal proportions	0.218	0.007	0.769	0.006
4Vs	Otoliths		0.140	0.195	0.624	0.041
	Alleles	Pseudo-Bayes	0.129	0.033	0.659	0.179
		Equal proportions	0.185	0.014	0.655	0.146
	Combined	Pseudo-Bayes	0.310	0.074	0.533	0.083
		Equal proportions	0.329	0.026	0.561	0.084

Note: The prior column refers to the prior used for the proportion for each allele for each locus in the base sample.

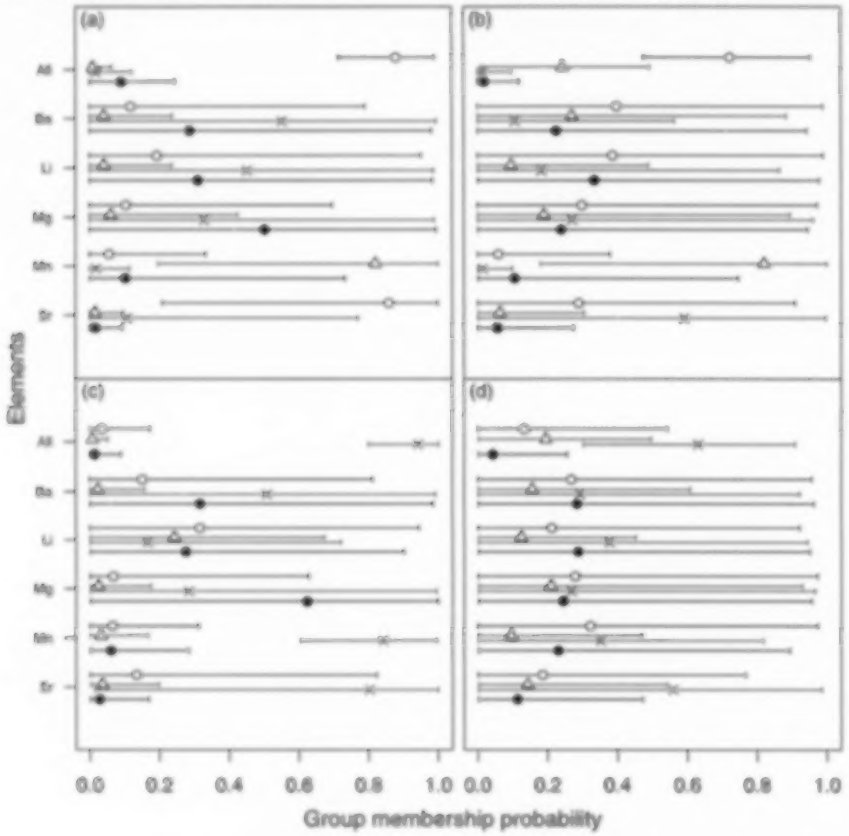
Table 3. Proportion of each 1997 mixed sample assigned to each of the 1997 base samples using either otolith elemental composition or allelic frequency data or both kinds of data combined.

			Base sample			
Mixed sample	Material	Prior	3Pn4RS	4T	4Vn	4Vs
3Pn4RS	Otoliths		0.953	0.038	0.005	0.004
	Alleles	Pseudo-Bayes	0.127	0.252	0.030	0.591
		Equal proportions	0.083	0.195	0.013	0.709
	Combined	Pseudo-Bayes	0.662	0.320	0.013	0.005
		Equal proportions	0.515	0.472	0.008	0.005
3Ps	Otoliths		0.963	0.006	0.009	0.022
	Alleles	Pseudo-Bayes	0.165	0.333	0.017	0.485
		Equal proportions	0.082	0.288	0.015	0.615
	Combined	Pseudo-Bayes	0.723	0.230	0.008	0.039
		Equal proportions	0.477	0.447	0.010	0.066
4T	Otoliths		0.018	0.937	0.028	0.017
	Alleles	Pseudo-Bayes	0.342	0.033	0.029	0.597
		Equal proportions	0.154	0.045	0.023	0.778
	Combined	Pseudo-Bayes	0.088	0.577	0.310	0.025
		Equal proportions	0.448	0.454	0.021	0.076
4Vn	Otoliths		0.014	0.907	0.070	0.009
	Alleles	Pseudo-Bayes	0.253	0.036	0.009	0.702
		Equal proportions	0.198	0.018	0.007	0.777
	Combined	Pseudo-Bayes	0.055	0.547	0.350	0.048
		Equal proportions	0.357	0.490	0.023	0.130

Note: The prior column refers to the prior used for the proportion for each allele for each locus in the base sample.

comparing the means of replicates from the posterior predictive distribution generated at each MCMC iteration with the original means for otolith elements from the base populations. Differences between original and new means were evaluated with a Bayesian version of Student's *t* test by evaluating the probability of obtaining a more extreme mean from the posterior relative to the original mean. We expect that this probability will be between 0.05 and 0.95 if the posterior distribution is a reasonable model for the base tows. Observed probabilities less than 0.05 would indicate

Fig. 2. Credible intervals (95%) for the posterior estimates of the proportions of cod in the mixed samples assigned to the different base groups using otolith elemental data from 1996. Credible intervals are coded by base groups: black line (open circle), 3Pn4RS; red line (triangle), 3Ps; green line (×), 4T; and blue line (solid circle), 4Vs. Position of posterior mean is indicated by symbols. Panels correspond to mixed samples (*a*) 3Pn4RS, (*b*) 3Ps, (*c*) 4Vn, and (*d*) 4Vs.



that the posterior mean was considerably smaller than was originally observed, whereas probabilities greater than 0.95 would imply the reverse situation.

The test statistic for the genetics data was based on χ^2 statistics for both replicate data from the posterior predictive distribution and the original allele frequency data for the base tows. That is,

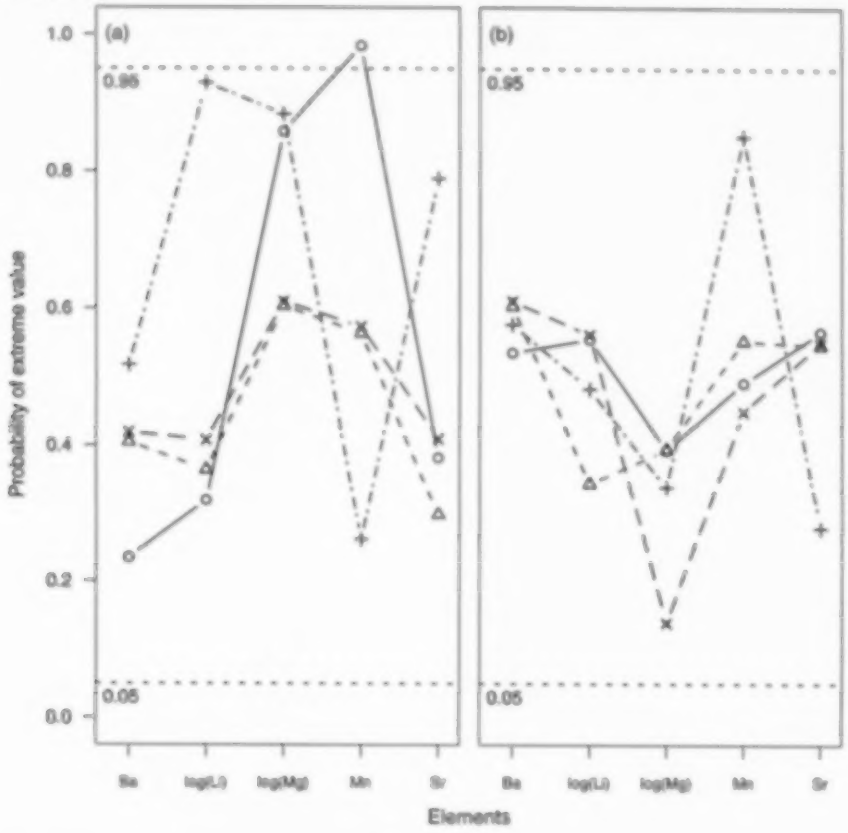
$$(3) \qquad X^2 = \frac{\sum_{k,j} (x_{k,j,b}^{rep} - N_b \theta_{k,j,b})^2}{N_b \theta_{k,j,b}}$$

where $x_{k,j,b}^{rep}$ is the replicated frequency of allele j at locus k from the current MCMC iteration from the posterior predictive distribution for total sample size N_b and base tow b. The probability associated with allele j from locus k, $\theta_{k,j,b}$, is also for the current iteration. The test statistic for the original frequencies for each loci for the base tows is constructed by substituting $x_{k,j,b}^{obs}$ for $x_{k,j,b}^{rep}$ in eq. 3. These test statistics are evaluated in terms of the probability that X_{rep}^2 is greater than or equal to X_{obs}^2 determined over a series of MCMC iterations. Probabilities less than 0.05 would be interpreted as indicating that the goodness-of-fit of the original observations to the posterior distribution was less than that for the combination of the base and mixed-sample observations. That is, the mixed-sample observations have modified the distribution such that they were more likely to arise from the posterior distribution than the base observations. When the probability was greater than 0.95, the base observations were more likely to have come from the posterior than from the combination of the base and mixed-sample observations.

Results

Classification of the 1996 mixed samples using otolith elemental composition resulted in the largest proportion of fish in the 3Pn4RS and 3Ps mixed samples being assigned to the 3Pn4RS base group, whereas cod in the other two mixed samples (4Vn and 4Vs) were mainly assigned to the 4T base group (Table 2). However, the results of the classification based on the frequency of alleles split the majority of the fish in the 3Pn4RS mixed sample between 3Pn4RS and 4T. The larger proportion of the 3Ps, 4Vn, and 4Vn mixed samples were assigned to 4T. Results were similar for either choice of prior for the allele frequencies. The combination of otoliths and alleles produced classifications closer to those of otoliths alone than those of alleles alone.

Fig. 3. Probability of observing a more extreme value for the mean of each of the otolith elements from the marginal posterior distribution relative to the distribution from the base samples in 1996: (*a*) classification of samples using all of the elements; (*b*) classification of samples using one element at a time. Bounds for 0.90 probability limits are indicated on each panel. Pseudo-Bayes prior is used for allele frequencies. Results are coded by base groups: solid line (open circle, 3Pn4RS; short-dashed line (triangle), 3Ps; dashed–dotted line (cross), 4T; and long-dashed line (×), 4Vs.



Again there was not a great deal of difference between the two priors for the allele frequencies.

There were more differences between otolith and genetic classification results for the 1997 data (Table 3). As was the case for the 1996 mixed sample, the majority of the fish from the 1997 mixed samples were assigned either to the 3Pn4RS group (3Pn4RS and 3Ps mixed samples) or to 4T (4T and 4Vn mixed samples) using the otolith data. The larger proportion of fish from all of the mixed samples was assigned to the 4Vs base group by the genetics data. The combination of otoliths and alleles resulted in only small proportions of the mixed samples being assigned to the 4Vs base group. For the model using the pseudo-Bayes prior for the allele frequencies, the larger proportion of mixed samples was assigned to the 3Pn4RS base group for the 3Pn4RS and 3Ps mixed samples, similar to the otolith only results. For the other two mixed samples, the larger proportions were assigned to the 4T and 4Vn base groups. However, the equal probability prior resulted in a similar split between the 3Pn4RS and 4T base groups for all of the mixed samples.

The credible intervals for the proportions assigned to the base samples in 1996 when using otolith elements alone indicate that only the proportion assigned to 3Pn4RS in the 3Pn4RS mixed sample and the proportion assigned to 4T from the 4Vn mixed sample differed significantly from proportions assigned to the other base groups at the 95% level (Fig. 2). When fish in the mixed samples were classified using each otolith element separately, the only case in which credible regions do not overlap was for the proportion assigned to 4T in the 4Vn mixed sample based on manganese. In contrast to the combined results, the only case in which a large proportion of cod was assigned to 3Pn4RS when individual elements were used was for strontium in the 3Ps4RS mixed sample.

The posterior predictive check on the mixture model for the 1996 data shows that the mean for manganese in the posterior distribution for 3Pn4RS was far lower than that observed for the original base group such that it is unlikely (probability of 0.016) that we would observe the original sample based on the parameters of the posterior (Fig. 3*a*). The means for lithium and magnesium for the 4T posterior distribution were also much lower than the means of the original base samples with associated probabilities <0.12.

Fig. 4. Credible regions (95%) for classification of mixed samples by base groups of allelic frequency data from 1996. Credible intervals are coded by base groups: black line (open circle), 3Pn4RS; red line (triangle), 3Ps; green line (×), 4T; and blue line (solid circle), 4Vs. Position of posterior mean is indicated by symbols. Panels correspond to mixed samples (*a*) 3Pn4RS, (*b*) 3Ps, (*c*) 4Vn, and (*d*) 4Vs.

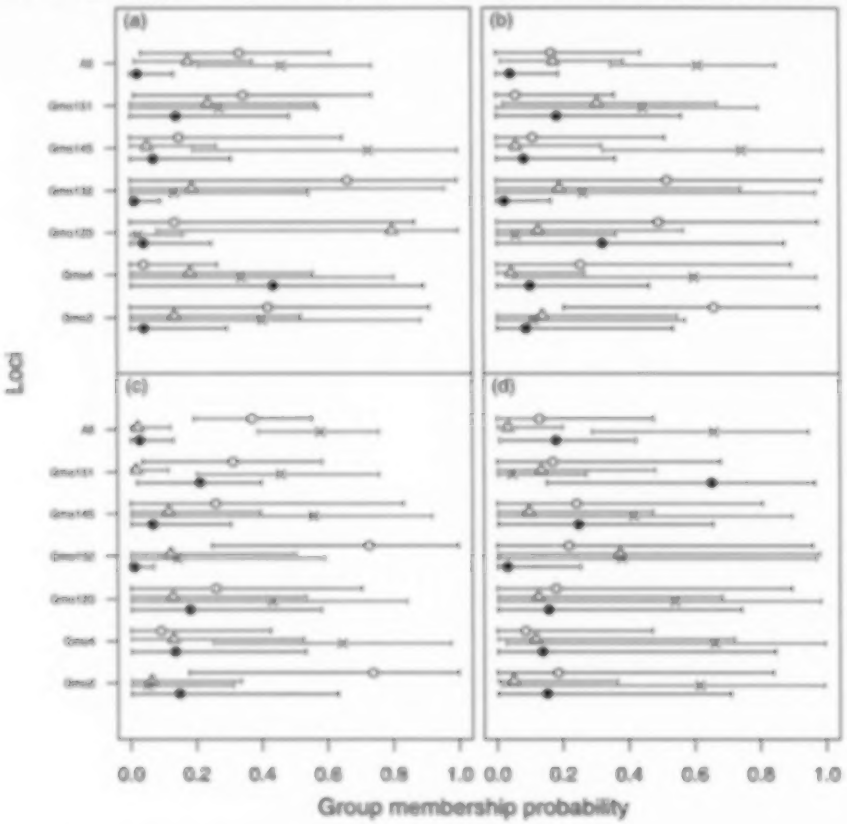


Table 4. Single locus and overall estimates of F_{ST} among the four base groups.

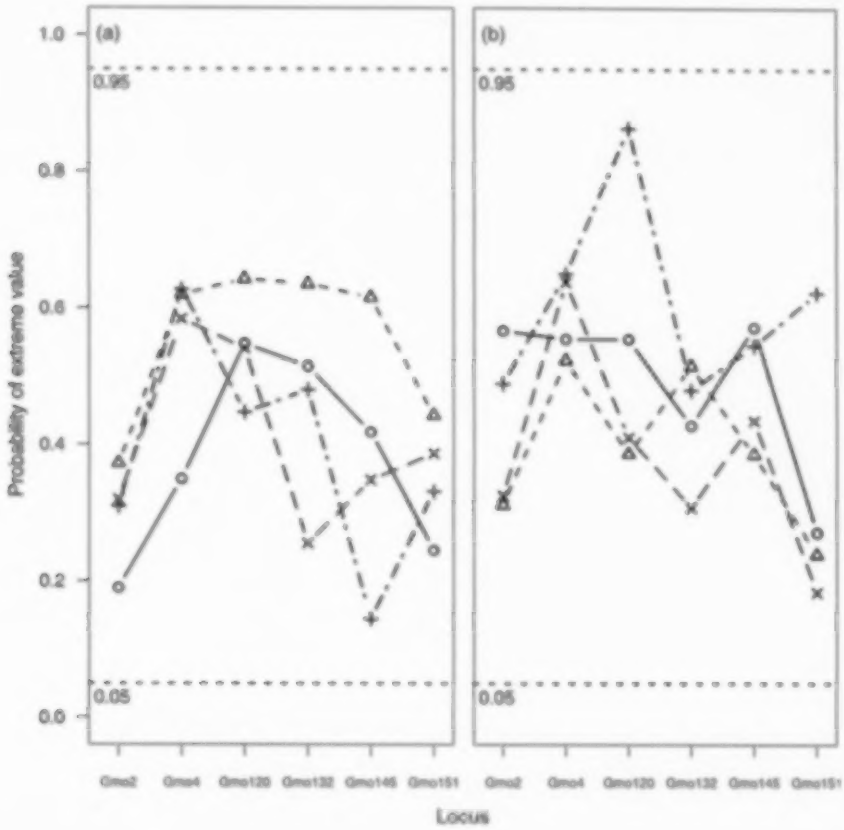
	Locus						
Year	Gmo2	Gmo4	Gmo120	Gmo132	Gmo145	Gmo151	Overall
1996	–0.0009	0.0001	–0.00001	0.0080*	0.0025	0.0037*	0.0020*
1997	–0.0011	–0.0007	–0.00060	0.0300*	0.0040	0.0103	0.0062*

Note: An asterisk indicates p level for test statistic < 0.0083 (= 0.5/6).

None of the posterior predictive probabilities was beyond the limits in Fig. 3*b* when classification was based on each element alone. The probability of exceeding the mean from the original base sample for manganese is now closer to 0.50 as more of the 3Pn4RS and 3Ps mixed samples were assigned to the 3Ps base group (Fig. 2). Comparison of the distribution of manganese for the base and mixed samples shows that the distributions for these two mixed samples were much closer to the 3Ps base sample than to the 3Pn4RS base to which they were assigned when all elements were used. Assignment to the latter base group for the all element case was due to strontium dominating the classification and resulted in large numbers of observations of manganese less than zero (detrended manganese) substantially lowering the mean for posterior distribution of manganese for 3Pn4RS relative to the original base group. Because Mn was the only element requiring statistical removal of a substantive effect due to fish length (Campana et al. 2000), it seems likely that the transformation somehow induced some unintended artifacts. This issue will be explored in later work.

The credible intervals for the proportions of the 3Pn4RS and 3Ps mixed samples assigned to the 3Pn4RS base group and of the 4T and 4Vn mixed samples assigned to the 4T base group do not overlap with the proportions assigned to the other base groups when using only otolith data in 1997. However, the classification results when using all elements appear to be the same as those for manganese alone as all other credible intervals overlap for the other elements. Despite the fact that manganese appears to be driving the classification when all elements are being used, the posterior predictive diagnostics indicate that the means of the poste-

Fig. 5. Probability of observing a more extreme value for the goodness-of-fit statistic for allele frequencies from the marginal posterior distribution relative to the distribution from the base samples in 1996: (*a*) classification using all loci; (*b*) classification using one locus at a time. Bounds for 0.90 probability limits are indicated on each panel. Pseudo-Bayes prior was used for allele frequencies. Results are coded by base groups: solid line (open circle), 3Pn4RS; short-dashed line (triangle), 3Ps; dashed-dotted line (cross), 4T; and long-dashed line (×), 4Vs.



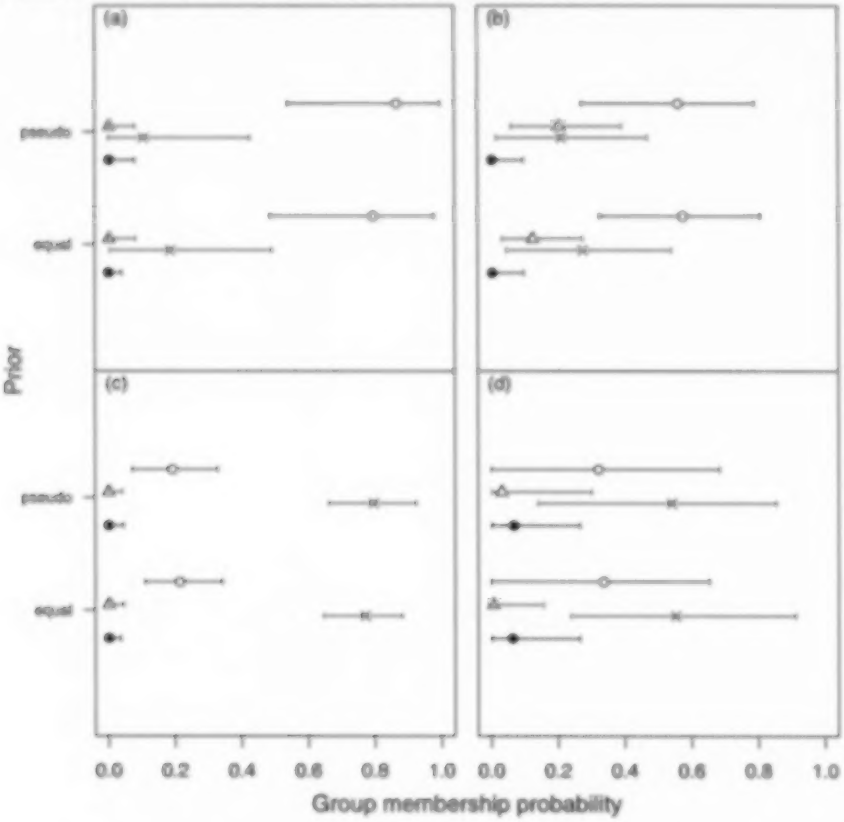
rior distribution for manganese are either higher than expected for the 4T base group or lower than expected for the 3Pn4RS base group. This pattern remains so for manganese even when classification is based on each element alone.

On the other hand, the larger proportion of cod in the 3Pn4RS and 3Ps mixed samples could be assigned to either the 3Pn4RS or the 4Vs base groups, resulting in a decreased mean for the posterior distribution for base group in either case. That the larger proportions of these mixed samples are assigned to 3Pn4RS may be due mainly to the much larger sample sizes in these mixed samples than in the base groups. Together these mixed samples account for 332 observations and can easily swamp the base groups for 3Pn4RS (47 observations) or 4Vs (82 observations) (Table 1). During the initial MCMC iterations, enough of the 3Pn4RS and 3Ps mixed-sample observations could be randomly assigned to the 3Pn4RS base group to lower the mean substantially such that all future iterations will assign the cod from these mixed samples to 3Pn4RS. The smaller sample size for the 3Pn4RS base group relative to the 4Vs base group may result in the former group being more vulnerable to this swamping effect. In fact, trials conducted with starting values for the group membership variable favouring the 4Vn base group for these mixed samples will result in the majority of their observations being assigned to the 4Vn base group. This base group has the smallest number of observations and can be more vulnerable to swamping if enough observations are are assigned to it in the initial iterations.

With the possible exception of the 4Vn mixed sample, the credible intervals all overlap for the classification when using only the allele frequencies in 1996 (Fig. 4). One could also argue that for a shorter interval corresponding to 90% coverage, the larger proportions assigned to 4T in the 3Ps and 4Vs mixed samples (possibly 4Vn as well) could well have credible intervals that do not overlap. However, the intervals for classifications based on a single locus overlap for all of the mixed samples.

The equivocal classification results were not unexpected given the low F_{ST} estimates and minimal genetic structuring observed among the four baseline groups analyzed (Table 4). The posterior predictive diagnostic indicates no serious issues with the allele frequencies for the posterior distribution when all loci are used (Fig. 5*a*) or when each individual locus is used (Fig. 5*b*).

Fig. 6. Credible intervals (95%) for the posterior estimates of the proportions of cod in the mixed samples assigned to the different base groups using both otolith elemental data and allele frequencies from 1996. Credible intervals are coded by base groups: black line (open circle), 3Pn4RS; red line (triangle), 3Ps; green line (×), 4T; and blue line (solid circle), 4Vs. Position of posterior mean is indicated by symbols. Panels correspond to mixed samples (*a*) 3Pn4RS, (*b*) 3Ps, (*c*) 4Vn, and (*d*) 4Vs.



The credible intervals for the proportion assigned to the 4Vs base group for the 3Pn4RS and 3Ps mixed samples were the only intervals not to overlap with the other intervals when only allele data were used to classify the mixed samples from 1997. The results for the 3Pn4RS mixed sample for all loci were very similar to those for locus Gmo145 alone, while classification was equivocal for the rest of the loci in this and the other mixed samples. Again the estimates of F_{ST} indicate little structure for the 1997 data (Table 4). There was no evidence of problems from the posterior predictive estimates when either all loci were used or each locus was used separately.

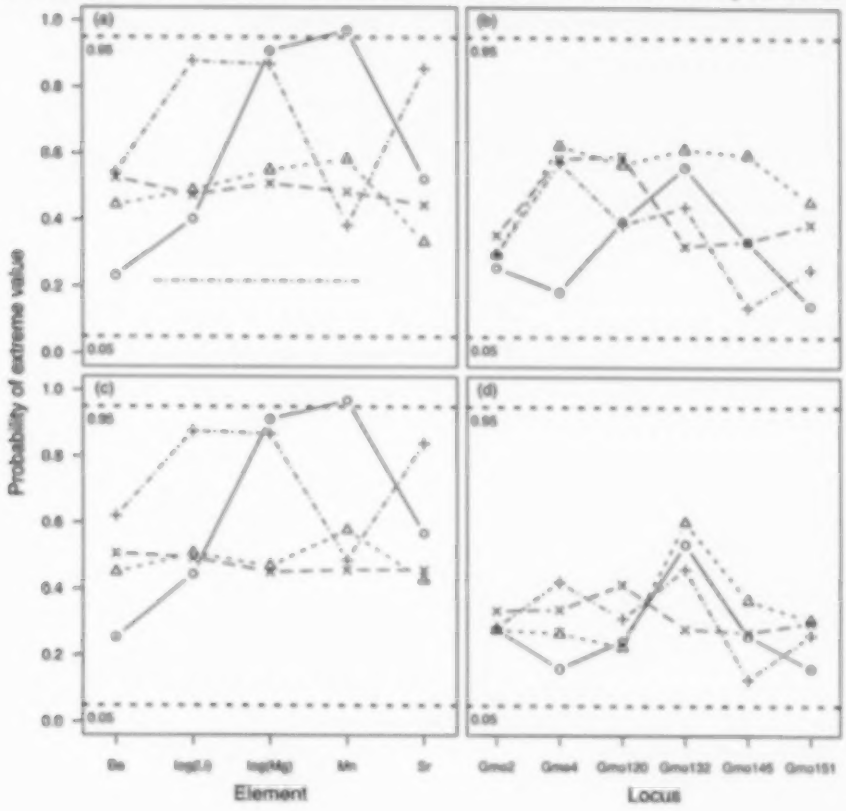
The credible intervals for the combined otolith and allele frequency data in 1996 were almost identical to those for otoliths alone (Fig. 6). There was very little difference in the intervals between the results from the pseudo-Bayes prior and the equal probability prior. The predictive posterior statistics changed little in pattern from the results when either otoliths or allele frequencies were used (Fig. 7).

Using both otoliths and allele frequency data for 1997 resulted in credible intervals indicating weaker support for classification of the larger proportions of the 3Pn4RS and 3Ps mixed samples to the 3Pn4RS and 4T base populations when using the pseudo-Bayes prior than when using otoliths alone. Credible intervals indicated equivocal classification for the 4T and 4Vn mixed samples for the pseudo-Bayes prior and for all mixed samples for the equal probability prior (Fig. 8). The difference between the means for manganese from the original and posterior base populations no longer appears to be an issue when either prior is used for the allele frequencies. However, the goodness-of-fit for loci Gmo120, Gmo132, Gmo145, and Gmo151 has declined as a result (Fig. 9).

Discussion

The data analyzed here represent a subset of the data sets analysed by Campana et al. (1999) and Ruzzante et al. (2000). The reduced data set was a consequence of choosing only cod for which both the otolith elemental composition and allele frequencies had been measured. The more-complete data sets contained more base populations and larger numbers of observations per base and mixed samples when considering only otolith or genetic measurements. In addition, Ruzzante et al. (2000) combined 1996 and 1997 allele frequency data, thus increasing sample sizes for many of the areas. A pooled-year approach was not appropriate for the

Fig. 7. Probability of observing a more extreme value for the mean of each of the otolith elements and for the goodness-of-fit statistic for allele frequencies from the marginal posterior distribution relative to the distribution from the base samples in 1996: (*a* and *b*) results from using the Pseudo-Bayes prior; (*c* and *d*) results from using the equal probability prior. All loci were used in the classification. Bounds for 0.90 probability limits are indicated on each panel. Pseudo-Bayes prior was used for allele frequencies. Results are coded by base groups: solid line (open circle), 3Pn4RS; short-dashed line (triangle), 3Ps; dashed–dotted line (cross), 4T; and long-dashed line (×), 4Vs.



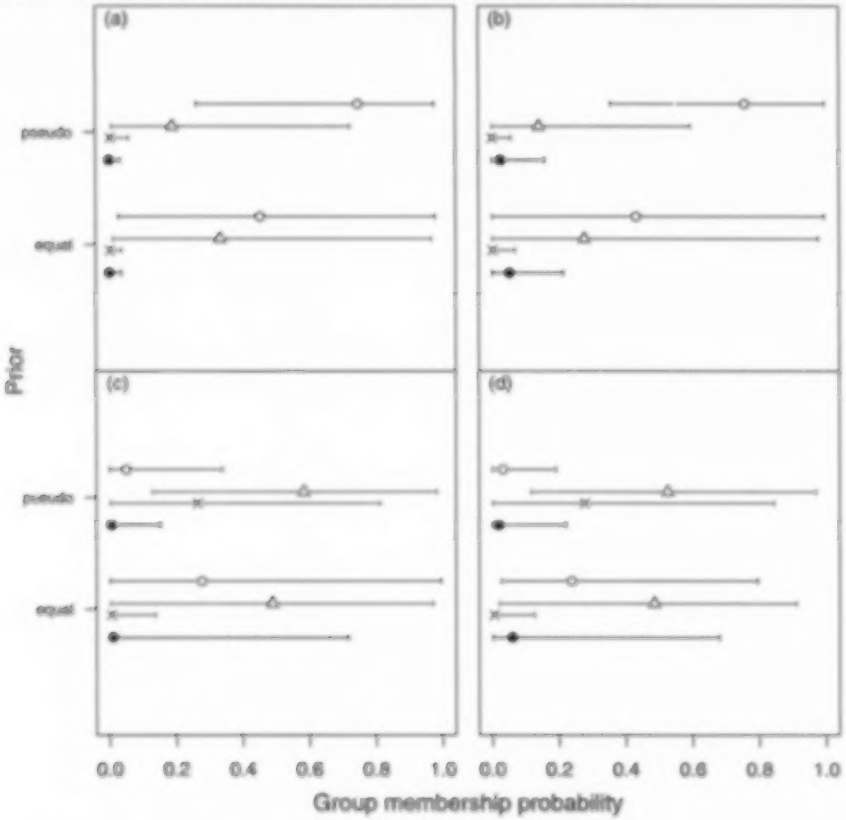
otolith data given that the elemental markers are not necessarily constant across years (Campana et al. 2000).

Despite these differences, our results from the otolith data are broadly comparable with those from Campana et al. (1999). The majority of cod from the mixed samples in 3Pn4RS were classified as belonging the 3Pn4RS base population in both years. Cod from 4T, 4Vn, and 4Vs mixed samples were mainly identified as being from the 4T base population. However, the larger proportion of cod from the mixed sample taken in 3Ps both years were classified as belonging to the 3Pn4RS population, whereas Campana et al. (1999) reported that these fish were classified as coming from 3Ps. The difference may be explained by the fact that our subset of the data contained fewer 3Ps base population records in 1996 and no records for the 3Ps base population in 1997. Confidence intervals were not reported in Campana et al. (1999).

Ruzzante et al. (2000) found that the larger proportion of all of the mixed samples was classified by the allele frequency data as coming from the 4T base population. Classification to the other base populations was equivocal based on the 95% bootstrap confidence intervals presented in their figs. 4 and 5. The only similar results in our case were for the 4Vn and 4Vs mixed samples in 1996.

The Gmo132 locus was identified here as exhibiting significant genetic differentiation. This locus has also been identified as exhibiting high genetic differentiation in cod stocks across the North Atlantic (Bentzen et al. 1996) and off the coast of Norway (Wennevik et al. 2008). This locus also had the least number of alleles of the loci used in our study and in those of both Ruzzante et al. (2000) and Bentzen et al. (1996). Although some authors suggest that the more polymorphic a locus is, the better able it is to differentiate between populations (Bernatchez and Duchesne 2000; Kalinowski 2004), others have found the opposite to be true (e.g., O'Reilly et al. 2004). Nielsen et al. (2006) has suggested that the higher genetic differentiation exhibited by this locus could be indicative of it being subject to hitchhiking selection. They also argue that although this would result in inflated levels of differentiation, these kinds of selected markers would also have increased statistical power for mixed-stock analysis compared with neutral markers. However, in our case, classification by this locus alone was equivocal in our analysis of both the 1996 and 1997 mixed samples.

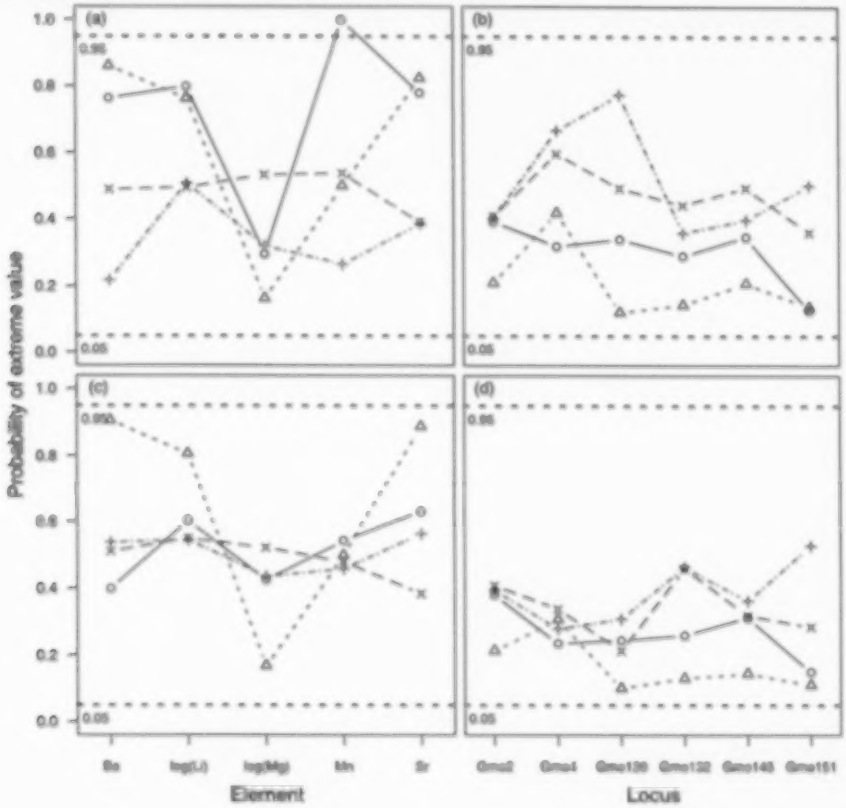
Fig. 8. Credible intervals (95%) for the posterior estimates of the proportions of cod in the mixed samples assigned to the different base groups using both otolith elemental data and allele frequencies from 1997. Credible intervals are coded by base groups: black line (open circle), 3Pn4RS; red line (triangle), 4T; green line (×), 4Vn; and blue line (solid circle), 4Vs. Position of posterior mean is indicated by symbols. Panels correspond to mixed samples (*a*) 3Pn4RS, (*b*) 3Ps, (*c*) 4T, and (*d*) 4Vn.



Our Bayesian credible intervals were wider than the bootstrap limits presented in Ruzzante et al. (2000). These 95% bootstrap limits were constructed by sampling with replacement from both the base and mixed populations a number of times and then obtaining the 0.025 and 0.975 quantiles of the maximum likelihood estimates from each sampling. Bolker et al. (2003) has also reported that Bayesian credible intervals tend to be wider than the bootstrap limits from the maximum likelihood stock composition model for a simulation study, as well as when analysing mitochondrial DNA from sea turtles. From their simulation study, they concluded that the Bayesian credible intervals were more robust than the bootstrap confidence intervals especially when many of the base populations contribute sparsely to some of the mixed samples.

Both Campana et al. (1999) and Ruzzante et al. (2000) used maximum likelihood conditional estimation for their stock composition analysis. As a result, they had limited means by which to evaluate the performance of their classification model. Here the combination of unconditional estimation and the Bayesian predictive posterior distribution provides a direct means for analysis of model performance once the classification of the mixed samples has been completed. For the 1996 otolith data, it appears that classification based on all of the elements results in a distorted distribution of manganese in the posterior distribution for the 3Pn4RS base population. This distortion does not occur when manganese is used by itself to classify the data. It appears that strontium may be driving the allocation of the large number of samples to the 3Pn4RS base group, and this classification was not supported by magnesium. On the other hand, manganese was identified as an issue for the 1997 data, but there, two different mechanisms were in play. First, the mixed samples had much larger sample sizes than the base population samples, and the manganese samples from the 3Pn4Rs and 3Ps mixed samples could overwhelm the parameter estimates for any of the base populations. Second, the distribution of manganese measurements in these two mixed samples were atypical compared with any of the base populations, possibly indicating that one or more actual base populations were missing from the analysis. The distribution of manganese in the 3Pn4Rs and 3Ps mixed samples more closely resembled that for the 3Ps base population in the 1996 data than the distribution in the 3Pn4RS base population in either 1996 or 1997. Recall that a 3Ps base population was not identified in the subset of the 1997 data used here, although they were present in the otolith study of Campana et al. (2000). Similarly, the larger

Fig. 9. Probability of observing a more extreme value for the mean of each of the otolith elements and for the goodness-of-fit statistic for allele frequencies from the marginal posterior distribution relative to the distribution from the base samples in 1997: (*a* and *b*) results from using the Pseudo-Bayes prior; (*c* and *d*) results from using the equal probability prior. All loci were used in the classification. Bounds for 0.90 probability limits are indicated on each panel. Results are coded by base groups: solid line (open circle), 3Pn4RS; short-dashed line (triangle), 4T; dashed–dotted line (cross), 4Vn; and long-dashed line (×), 4Vs.



proportion of the mixed samples from 4T and 4Vn in 1997 was allocated to the 4T base group, but the posterior mean was also distorted compared with that of the base population. Again, disparate sample sizes were probably an issue, but this pattern may also point to a case of missing base populations in the analysis. Methods for classification when base populations are missing are presented in Corander et al. (2006), Pella and Masuda (2006), and White et al. (2008).

Our analysis integrating otolith and genetic markers did not result in enhanced classification success relative to either method by itself, although it produced classifications similar to those of the otoliths. The explanation for this perhaps counterintuitive result appears to be linked to the poor discriminatory power of the genetic markers for cod in and around the Gulf of St. Lawrence. Presumably, the poor genetic discrimination was due to the occasional mixing of cod spawners among the populations, in keeping with previous tagging results (Taggart et al. 1995). The integration of the weaker genetic discriminations with the stronger otolith-based classifications then degraded the overall classification success. Needless to say, this pattern is not necessarily representative of other population mixtures, where genetic assays may well provide superior discriminatory power over other methods. In this particular system, however, the genetic assays were not particularly informative in discriminating among populations.

The possibility that the different time scales represented by the genetic and otolith elemental techniques contributed to some inconsistencies between their results seems unlikely but cannot be excluded. Population-level differences in genetic markers usually evolve over many generations, whereas otolith elemental differences reflect environmental differences during the lifetime of the individual. In most situations, one would expect genetic differences also to be reflected in environmental differences due to the different habitats being occupied by each population (Begg and Waldman 1999). Although the reverse is not necessarily true, there is no reason to expect incompatibilities between the methods. In principle, as long as there are significant and stable differences in population markers among each of the source groups, the nature of the marker is irrelevant. In a marine ecosystem, genetic markers usually have the advantage of being stable across cohorts, whereas environmentally driven markers such as otolith elemental fingerprints usually have the advantage of producing larger differences among

groups of fish. The key to using them both in an integrated analysis is that known-origin reference groups exist for each.

A number of different materials and methods have been used on their own for stock identification (e.g., mark–recapture, parasites, otolith characteristics such as shape and composition, genetic material including mitochondrial and nuclear DNA), with each having its own assumptions and shortcomings. Begg and Waldman (1999) have recommended that more robust stock identifications could be obtained if using more than one kind of these materials provided similar stock identifications. On the other hand, issues arising from only using one method would be evident if the different methods resulted in different stock affinities. Although this recommendation is important to keep in mind when trying to identify putative base stocks, it is critical to the evaluation of the identification of the proportion of each base stock in a mixed sample.

We know of three published studies in which more than one kind of stock identification data were used to identify the proportion of base groups in mixed samples. In all three cases, different methods were used to analyse the different kinds of data. Bradbury et al. (2008) reported a conflict between the results of a maximum likelihood stock composition analysis of allele frequency data and a discriminant analysis of otolith elemental composition data for resolving the degree of early life history dispersal of the anadromous rainbow smelt, *Osmerus mordax*, in southeastern Newfoundland. In a second study, base populations were defined by otolith shape analysis or by a geographic criterion in Wennevik et al.'s (2008) analysis of the distribution of coastal and oceanic Atlantic cod (*Gadus morhua* L.) on the Lofoten spawning grounds, northern Norway. These authors used the BAYES computer program (Pella and Masuda 2001) to analyse the allele frequency data but simply tabulated the proportion of coastal-type or oceanic-type otoliths in the mixed samples. Thorrold et al. (2001) used the conditional maximum likelihood approach on otolith elemental composition data to estimate the proportion of adult weakfish (*Cynoscion regalis*) spawning in their natal estuary based on the base population of juveniles in each estuary. Genetic data were available but were not used in the analysis because a separate study concluded that there were no significant differences between populations based on an analysis of molecular variance (published in Cordes and Graves 2003). Both Thorrold et al. (2001) and Cordes and Graves (2003) suggested that there was sufficient exchange among the base populations (estuaries) to prevent genetic divergence.

Our framework allows for the evaluation of classifications based on one or more concurrently measured stock identification data. Our Bayesian model allows for the same base populations to be used to classify the same mixed samples for these different kinds of data. For example, the question about limited dispersal of rainbow smelt (Bradbury et al. 2008) could have been more precisely answered by using our model and the same base populations to analyse both the genetic and otolith data. The cod otolith shape data (Wennevik et al. 2008) could have been modelled as a multinomial random variable and analysed both separately and combined with the allele frequency data to investigate the distribution of the two base populations in the mixed samples. Finally, a combined analysis of the otolith and genetic data for the weakfish (Thorrold et al. 2001) could provide insights into the distributions of the genotypes assuming that the otolith data dominates the classification of the base groups. In each of these examples, a more powerful analysis of the available data would have been possible using an integrated statistical analysis.

The ready availability of Bayesian credible limits for separate or combined analysis of stock identification material allows for the assessment of the degree of uncertainty with respect to stock composition. These limits could in turn provide the basic information for assessments of the risk of using the resultant classifications when developing harvest advice or rebuilding plans for depleted or at-risk stocks. Similarly, the diagnostic tests presented here offer the means to evaluate the assumptions of the analysis such as identification of all of the base populations. The identification of missing base populations is an ongoing problem in stock composition analysis (Pella and Masuda 2006). Although this problem is not completely solved in this framework, the diagnostic tests do provide a valuable tool for recognizing the existence of missing base populations.

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Energy dynamics and growth of Chinook salmon (*Oncorhynchus tshawytscha*) from the Central Valley of California during the estuarine phase and first ocean year

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Abstract: The greatest rates of energy accumulation and growth in subyearling Chinook salmon (*Oncorhynchus tshawytscha*) occurred during the first month following ocean entry, supporting the importance of this critical period. Data from an 11-year study in the coastal ocean off California and the San Francisco Estuary revealed that juvenile salmon gained 3.2 kJ·day^{-1} and 0.8 g·day^{-1}, representing 4.3%·day^{-1} and 5.2% day^{-1}, respectively, relative to estuary exit values. Little gain in energy (0.28 kJ·day^{-1}) or size (0.07 g·day^{-1}) occurred in the estuary, indicating that the nursery function typically ascribed to estuaries can be deferred to initial ocean residence. Calculated northern anchovies (*Engraulis mordax*) equivalents to meet energy gains were one anchovy per day in the estuary (8% body weight·day^{-1}) and about three per day immediately following ocean entry (15% body weight·day^{-1}). Energy content in the estuary was positively related to higher salinity and lower freshwater outflow, whereas in the ocean, cooler temperatures, lower sea level, and greater upwelling resulted in greater gains. These results suggest that greater freshwater flows, warmer sea temperatures, and reduced or delayed upwelling, all of which are indicated by some (but not all) climate models, will likely decrease growth of juvenile Chinook salmon, leading to reduced survival.

Résumé : Les taux les plus importants d'accumulation d'énergie et de croissance chez les saumons chinook (*Oncorhynchus tshawytscha*) de moins d'un an ont lieu durant le premier mois qui suit leur entrée dans l'océan, ce qui souligne l'importance de cette période critique. Des données tirées d'une étude de 11 années dans l'océan côtier au large de la Californie et de l'estuaire de San Francisco indiquent que les jeunes saumons ont accumulé 3,2 kJ·jour^{-1} et 0,8 g·jour^{-1}, ce qui représente respectivement 4,3 %·jour^{-1} et 5,2 %·jour^{-1}, par rapport aux valeurs à la sortie de l'estuaire. Il se produit peu de gains en énergie (0,28 kJ·jour^{-1}) ou en taille (0,07 g·jour^{-1}) dans l'estuaire, ce qui veut dire que la fonction de nourricerie typiquement assignée aux estuaires doit être attribuée plutôt à la résidence initiale dans l'océan. Les équivalents calculés en anchois du Pacifique (*Engraulis mordax*) nécessaires pour expliquer les gains d'énergie sont d'un anchois par jour dans l'estuaire (8 % de la masse corporelle·jour^{-1}) et environ trois par jour immédiatement après la pénétration dans l'océan (15 % de la masse corporelle·jour^{-1}). Le contenu énergétique de l'estuaire est en relation positive avec une salinité élevée et un apport réduit d'eau douce, alors que dans l'océan, les températures plus fraîches, le niveau plus bas de la mer et les résurgences plus importantes entraînent des gains plus marqués. Nos résultats indiquent que des apports plus considérables d'eau douce, des températures de la mer plus élevées et des résurgences réduites ou retardées, tous des phénomènes signalés dans certains modèles climatiques (mais pas tous), vont vraisemblablement réduire la croissance des saumons chinook et mener à une diminution de la survie.

[Traduit par la Rédaction]

Introduction

It is generally considered that the estuarine and early ocean phases of salmonids' lives are critical periods for growth and survival (Pearcy 1992; Quinn et al. 2005; Beamish et al. 2008). Increased feeding (Healey 1991; Morgan et al. 2006) and predator avoidance (Simenstad et al. 1982) during these times allow juvenile salmonids to grow to sizes that confer survival advantages in the ocean (Pearcy 1992; Beamish and Mahnken 2001). Assessing somatic growth and stored energy accumulation offers a direct method of evaluating the physiological status, and thus survival potential, of outmigrating populations. This raises the question: what are typical growth and energy dynamics for juvenile salmonids during the estuarine and early ocean life phases? Without baseline data on the temporal pattern of growth and energy dynamics, the influences of natural or anthropogenic factors cannot be understood.

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Most studies of energy dynamics in Pacific salmon have concerned expenditures of returning adults to the spawning grounds, and most of these studies were for sockeye salmon (*Oncorhynchus nerka*), chum salmon (*Oncorhynchus keta*), and pink salmon (*Oncorhynchus gorbuscha*) (reviewed in Brett 1995; Rand et al. 2006). Far fewer data are available for juvenile salmonids, including Chinook salmon (*Oncorhynchus tshawytscha*). There have been investigations into aspects of juvenile Chinook salmon growth as a function of environmental conditions, e.g., temperature, ration, and photoperiod (Banks et al. 1971; Clarke et al. 1981; Brett et al. 1982), but these have been mostly for the freshwater fry stage, not the older smolt or ocean juvenile stages. From an energy perspective, Trudel et al. (2005) assessed energy density of juvenile Chinook salmon and found that energy density was primarily a function of lipid and, to a lesser degree, protein content, but temporal patterns were not explored during estuarine use and initial ocean entry.

Although there are a few studies of juvenile Chinook salmon growth in estuaries (Reimers 1973; Healey1982; Levy and Northcote 1982), there are even fewer during the first year in the ocean (Fisher and Pearcy 1995), particularly for ocean-type Chinook salmon. Previous studies have concluded that juvenile ocean-type Chinook salmon utilize estuarine habitats extensively to feed and grow (Simenstad et al. 1982; Healey 1991). Synoptic assessments of the same population through the estuarine and first ocean year phases of their life cycle do not exit (Healey 1991; Weatherly and Gill 1995).

Establishing a time course of somatic and energy growth that is representative of juvenile Chinook salmon through the estuarine and first ocean year is timely given the increasing evidence of climate-mediated ocean environmental changes (Pierce 2004; Sarmiento et al. 2004). Abundant recent research has linked trends in Pacific salmon survival to changes in ocean conditions (Coronado and Hilborn 1998; Koslow et al. 2002; Beamish et al. 2004), with early ocean mortality hypothesized to be the primary factor (Hare et al. 1999; Beamish and Mahnken 2001). By relating changes in energy content in juvenile Chinook salmon to specific environmental variables in the estuary and in the ocean, increased understanding is gained as to which factors may be exerting influence and, thus, how climate-induced changes to these variables may affect salmon growth and subsequent survival.

The purpose of this paper is to present information quantifying changes in size and energy content of subyearling juvenile Chinook salmon from estuary entry through the first ocean winter. In an 11-year study, the population from California's Central Valley, which emigrates through the San Francisco Estuary into the coastal ocean off northern California, was sampled to gather data on these processes. These data are used to test the hypothesis that somatic (i.e., muscle protein) growth predominates during the estuarine phase to acquire larger size, thus conferring greater survival potential in the ocean, whereas during the early ocean period, the balance of energy accumulation shifts toward a greater proportion of stored energy (i.e., triacylglycerols) to sustain life through the first winter, when prey resources are less abundant (Ainley 1990). To identify factors influencing bioenergetic processes, interannual variability of growth and energy dynamics within the estuarine and the early ocean phases was related to environmental variables. From previous work by Botsford and Lawrence (2002) and Wells et al. (2007, 2008), I hypothesized that increased upwelling and cooler sea surface temperatures resulted in greater gains in total energy content during the early ocean phase, but zooplankton abundance was linked to gains in the estuary. Finally, an estimate of prey ration required to achieve mean changes in energy content within the estuary and during the summer, fall, and winter of the first ocean year was calculated.

Material and methods

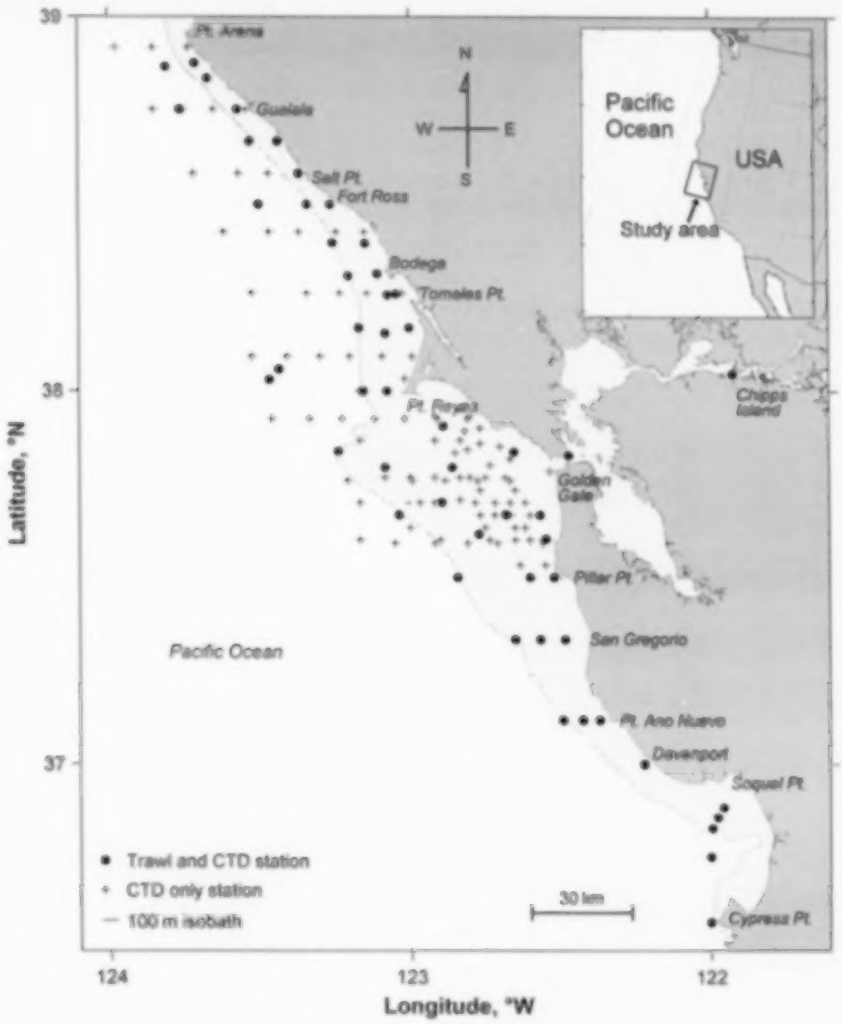
Field sampling

Salmon collection

Ocean-type subyearling Chinook salmon were sequentially sampled temporally and spatially as they emigrated from natal sources in the Central Valley of California through the estuary and into the coastal ocean. Juvenile Chinook salmon were collected at two locations within the San Francisco Estuary: at Chipps Island, the head of the saline estuary at the confluence of the Sacramento and San Joaquin rivers, and at the estuary exit at the Golden Gate (Fig. 1). To target the fall run, by far the most abundant of the four Chinook salmon runs in California's Central Valley, sampling in the estuary was conducted in mid-April through June from 1995 through 2005 (Fig. 2). These months are when the vast majority of salmon transit the estuary (Kjelson et al. 1982; Fisher 1994). Juvenile Chinook salmon of all four runs from rivers and streams in California's Central Valley emigrate through the San Francisco Estuary and enter the coastal ocean between October and July (Fisher 1994) primarily as subyearlings, although there are small percentages of the spring and late-fall cohorts that emigrate as yearlings (Clark 1929; Calkins et al. 1940; Fisher 1994). Collections were made with a midwater trawl towed at the surface at 2–3 kn for 15–30 min. The trawl sampled the top 10 m of water, had head and foot ropes of 10 m, and was 20 m long. Nylon mesh was 1.6 cm at the head rope and decreased to 0.4 cm at the codend. The codend was fitted with a 1.27 cm knotless mesh liner. Details of the trawling process are in MacFarlane and Norton (2002).

Cruises were conducted in the coastal ocean between 1995 and 2005. Sampling occurred during three seasons, the dates and durations determined by ship availability. A combination of the NOAA ship *David Starr Jordan* and chartered commercial trawlers was used to collect juvenile salmon. The experimental design called for cruises in the summer, fall, and winter to follow salmon dynamics within a month or so of ocean entry (summer), a couple months later (fall), and then in the winter of their first ocean year (winter), but lack of vessel availability or funding resulted in only the summer and fall schedule being completed in all years (Fig. 2). Fish were sampled with a Nordic 264 rope trawl towed at 3–4 kn at the surface for 6–40 min (mean = 22 min), inversely related to the density of *Chrysoara fuscescens* jellyfish. The net was about 27 m wide and 194 m long and fished the top 14 m of water, based on depth loggers added to the head and foot ropes. Mesh size graded from 163 cm in the throat of the trawl to 9 cm in the

Fig. 1. Locations of estuarine and ocean stations where juvenile Chinook salmon (*Oncorhynchus tshawytscha*) and oceanographic data were collected.



codend. The codend liner was 6 × 10 mm knotless nylon. More details of the trawling operation can be found in MacFarlane et al. (2005). Trawl stations were located between Cypress Point (36°35′N, 121°58′W) and Point Arena (38°57′N, 123°44′W) in water depths of 50–150 m (Fig. 1).

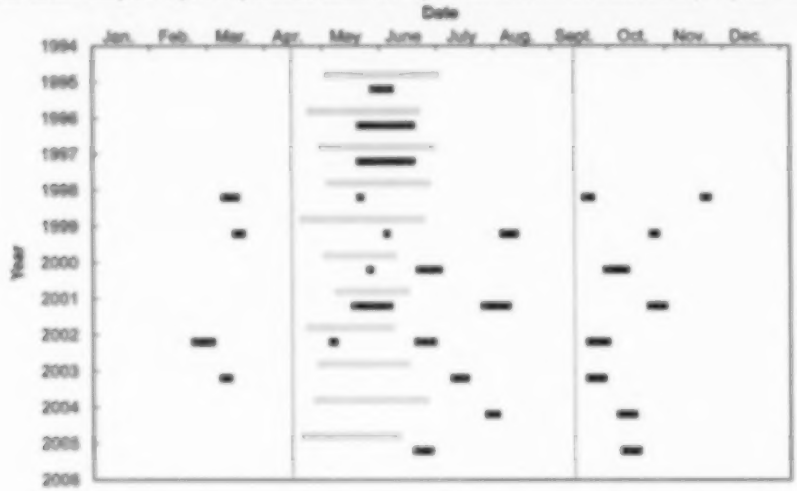
For both estuarine and coastal trawls, juvenile salmon were removed from the catch and stored either at –80 °C (coastal) or under ice (estuary) until they were returned to the laboratory for later examination and dissection. When iced, fish were transferred to –80 °C after no more than 4 h.

Environmental data

In addition to trawls, plankton samples and oceanographic data were acquired at each station and at numerous other stations in the coastal ocean to characterize the water over the shelf in the study area. Plankton were collected by Tucker trawl; neuston were collected by Manta net. Depth profiles of oceanographic data were acquired by using conductivity–temperature–depth (CTD) instruments (Sea-Bird Electronics, Inc., Bellevue, Washington), and when using the NOAA ship *David Starr Jordan*, surface values of temperature, salinity, and chlorophyll fluorescence were recorded continuously by the ship's thermosalinometer. Plankton and oceanographic data procedures are described in more detail in MacFarlane et al. (2005).

To get a broader spatial and temporal representation of hydrologic conditions in the estuary and oceanographic conditions in the coastal ocean, data were acquired from various sources. Estuarine temperature, salinity, density, oxygen concentration, and chlorophyll fluorescence data were means of total water column measurements taken in May and June of each year at 18 stations on a transect from the confluence of the Sacramento and San Joaquin rivers to the Golden Gate (US Geological Survey, http://sfbay.wr.usgs.gov/access/wqdata/index.html). Freshwater outflows to the estuary were the means of daily flow for May and June derived

Fig. 2. Collection schedule for juvenile Chinook salmon (*Oncorhynchus tshawytscha*) between 1995 and 2005. Shaded bars are estuarine cruises, solid bars are ocean cruises. Estuarine sampling was done in May and June of each year. Calendar breaks in April and September delimit the time spans for ocean samples: April–September, summer; September–December, fall; January–April, winter.



from gauging stations in the Central Valley (California Department of Water Resources, http://iep.water.ca.gov/dayflow/). Sea surface temperatures were means of monthly means for May to September from composite satellite images for the coastal area (shore to approximately 200 m offshore) bounded by 36°30′N and 38°40′N latitude (NOAA Fisheries, Southwest Fisheries Science Center, Environmental Research Division, http://www.pfeg.noaa.gov/products/las.html). Upwelling indices were the means of daily means for May to September for data from 36°N and 39°N (NOAA Fisheries, Southwest Fisheries Science Center, Environmental Research Division, http://www.pfeg.noaa.gov/products/las.html). Freshwater outflows from the estuary were the annual means of daily flow for May through September derived from the Central Valley (California Department of Water Resources, http://iep.water.ca.gov/dayflow/). Sea level height data were the means of monthly means from May through September at the mouth of San Francisco Estuary (Ft. Point, 37°48′N, 122°28′W; University of Hawaii, http://ilikai.soest.hawaii.edu/uhslc/htmld/d0551A.html). Pacific Decadal Oscillation (PDO) data were annual means of monthly values (http://jisao.washington.edu/pdo/).

Laboratory analyses

Growth

In the laboratory, juvenile salmon were measured, examined, and dissected. Analyses occurred within 1 to 2 days after capture in the estuary and within a week of the end of ocean cruises, which typically lasted 5 to 7 days. Fish were measured for fork length and weighed. Condition factor (K) was calculated as

$$K = (W/L^3) \times 10^5$$

where W is weight (in grams) and L is fork length (FL, in millimetres). The peritoneal cavity was opened by incision from vent to opercular isthmus, and the stomach was removed and preserved in 10% buffered formalin for subsequent content analysis. Visceral fat was removed from the stomach prior to preservation and placed back into the peritoneal cavity. Sagittal otoliths were excised, prepared for age and growth analysis, and analyzed according to MacFarlane et al. (2005). Individual fish, minus the otoliths and stomachs, were placed in a Whirl bag, purged with N_2, and stored at –80 °C in the dark for later lipid and protein analyses.

Energy content

Energy content was determined by analyzing lipid classes and total protein concentrations in whole animals, multiplying those values by the animal's weight, and then summing all components. All organic tissue components are expressed as milligrams per gram wet weight. For conversion to dry weight equivalence purposes, juvenile salmon body water concentration is about 800 mg·g^{-1}.

Lipids were analyzed from a whole-fish homogenate. Heads and fins were removed and the partially thawed fish were homogenized in a Waring blender for up to 30 s to form a uniform paste. Total lipids were extracted from 1 to 3 g of paste by the chloroform–methanol biphasic procedure of Bligh and Dyer (1959). The concentrated extract was analyzed for steryl–wax esters, triacylglycerols (TAG), nonesterified fatty acids (NEFA), cholesterol, and polar lipids using thin-layer chromatography on Chromarods and flame-ionization detection by an Iatroscan TH-10 Mark V according to the methods in MacFarlane and Norton (1999). Total lipid concentration is the sum of the five lipid class concentrations. Total protein was determined on an aliquot of the whole-fish paste by the Lowry method using bovine serum albumin as the protein standard (Lowry et al. 1951).

Because only a proportion of fish were aged (1083 of 4907), I estimated age in days after hatch ($\widehat{A}$) for all juvenile salmon using the following regression:

$$\widehat{A} = 1.068L + 54.74 \quad (r^2 = 0.80, P < 0.001)$$

where L is as defined above. The agreement between calculated age and age by otolith analysis for the 1083 aged fish was 0.99 of otolith age ($r^2 = 0.98$), indicating that it is a valid estimator of otolith-derived ages.

To determine energy content, I used the values in Brett (1995) for protein (20.1 kJ·g^{-1}) and lipid (39.5 kJ·g^{-1}). Energy content was determined by multiplying the concentration of the component (total protein, total lipid, TAG) by the weight of the fish and then by the energy equivalency for the component. Total body energy content is the sum of total lipid and total protein contents.

Data were organized into five sampling locations or times: estuary entry, estuary exit, ocean (summer), ocean (fall), and ocean (winter) to facilitate comparisons across years and locations or times. Changes in size or energy content within each location–time interval were expressed as a linear function. Changes within each interval when expressed as a percentage change per day were expressed as an exponential function, e.g.,

$$G = ((\ln W_{t_2} - \ln W_{t_1})/(t_2 - t_1)) \times 100$$

where G represents percentage change in weight (or energy content) within an interval between the beginning (t_1) and end (t_2) of a location–time interval.

Differences among locations or times and years for size, K, protein and lipid class concentrations, and energy content were determined by analysis of covariance (ANCOVA) using calculated age as the covariate. All statistical analyses were performed with SAS software (SAS Institute Inc., Cary, North Carolina).

Prey consumption estimation

From the size data at the beginning and end of each location–time interval, the ration necessary to account for the gains seen in each interval was estimated. A series of bioenergetic simulations were used to explore the consumption of prey, northern anchovies (*Engraulis mordax*), a primary food item for juvenile Chinook salmon (Brodeur and Pearcy 1990; Hunt et al. 1999; Schabetsberger et al. 2003), needed to fuel the observed growth. This was accomplished using an individual-based mass-balance bioenergetics model with a daily time step (Fish Bioenergetics 3.0; Hanson et al. 1997). These simulations were conducted using Chinook salmon energy densities determined by the mean of the sum of lipid and protein energy contents for fish collected in each location–time interval, a constant prey energy density of 311 kJ·g^{-1}, and estimating consumption by fitting the model to starting and final juvenile salmon weights. Temperatures used in each simulation were mean values for each location–time interval during the 11-year study. Because energy density data for *E. mordax* were not available, I used data for a closely related congener, *Engraulis encrasicolus* (Grant et al. 2005; Tirelli et al. 2006). Size of northern anchovies for each estuarine and ocean period were rounded values (nearest 5 mm standard length, SL) from stomach contents (R.B. MacFarlane, unpublished data). Anchovy weight (grams) was determined by the relationship:

$$W = (0.40 \times 10^{-5})L^{3.2}$$

(Messersmith 1969).

Environmental influences on energy content

Changes in energy content of juvenile salmon between estuary entry and exit and between estuary exit (ocean entry) and the ocean (fall) collections were related to environmental variables by principal components analysis (PCA) in SAS. Variables were chosen according to those shown to relate to growth in Chinook salmon (Botsford and Lawrence 2002; Wells et al. 2007, 2008) or perceived to be important to salmon ecology. For estuarine influences, water temperature (temp), salinity, density (σ_t), oxygen concentration (O_2), chlorophyll *a* concentration (chloro), freshwater outflow rate (outflow), and zooplankton concentration (plankton) were evaluated. In the ocean, sea surface temperature (SST), sea level height, upwelling index, freshwater outflow, Pacific Decadal Oscillation (PDO), and zooplankton concentration were used.

Results

During the 11 years of the study, a total of 4907 juvenile Chinook salmon were collected, of which 1479 were subjected to lipid and protein analysis. Ocean samples in the fall and winter were less abundant than those from the estuary and ocean in the summer because juvenile Chinook salmon are more widely dispersed and likely more capable of avoiding the net due to their larger size. Also, in some cases, ship time was not available in the fall and winter. Although interannual comparisons are not the subject of this paper, annual mean values for size, age, lipid and protein concentrations, and total energy content at each location or sampling time are provided (Appendix A, Table A1).

Growth

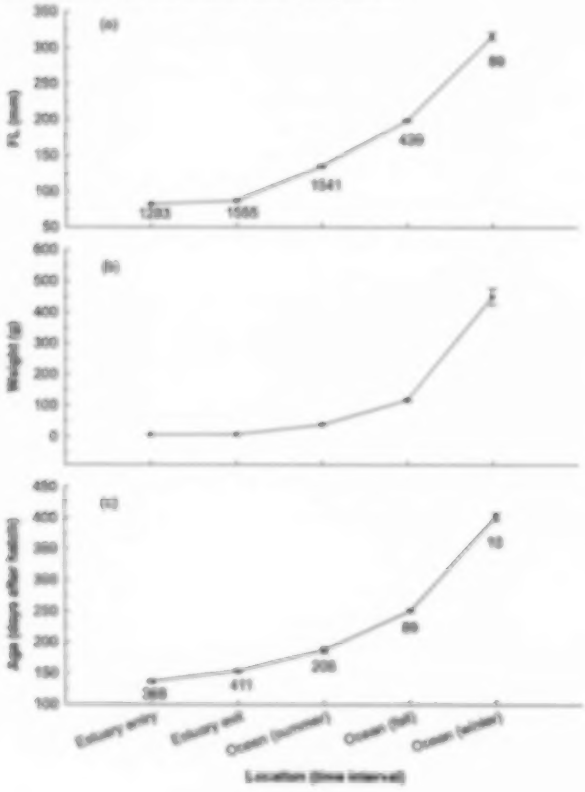
As expected, there was statistically significant growth ($P < 0.0001$) between sampling locations or times when combining data from all years. Further, there were significant differences among years at each location in mean FL and weight ($P < 0.0001$), except for weight in the estuary, which was not significantly different between estuary entry and exit among years. Juvenile Chinook salmon enter the estuary at 137 ± 1 days old (mean ± standard error, SE) and transit the 68 km length of the estuary in about 19 ± 3 days, averaging about 0.5 body lengths·s^{-1}, based on the mean FL at estuary entry of 82.5 ± 0.2 mm (Fig. 3). Little growth occurred while in the estuary, but both FL and weight increased rapidly once juveniles entered the ocean (Table 1). At estuary exit, or ocean entry, Chinook salmon were 87.1 ± 0.3 mm FL and weighed 7.4 ± 0.1 g, which represents growth of 0.33 ± 0.1 mm·day^{-1} and 0.07 ± 0.03 g·day^{-1} while in the estuary. During the first 29 days in the ocean, growth was the fastest of any location–time interval (Table 1). On a basis of percentage increase per day from estuary exit, juveniles gained 1.25% FL·day^{-1} and almost 5.2% weight·day^{-1} in the first month at sea. The greatest absolute growth rate occurred in the period between the summer and fall (65.6 days). By fall, the typical juvenile Chinook salmon was 198 ± 1 mm FL and weighed 117.2 ± 2.2 g, which translates into growth of 1.19 ± 0.13 mm·day^{-1} and 1.47 ± 0.16 g·day^{-1} (Fig. 3). Growth slowed between fall and winter. Over the 159-day interval, salmon grew at the rate of 0.51 ± 0.26 mm·day^{-1} and 1.13 ± 0.74 g·day^{-1}.

Table 1. Calculated mean of annual means for rates and changes within each location–time interval of growth and energy content of juve-

		ΔFL		ΔWeight		ΔProtein
Interval	No. of days	mm·day^{-1}	%·day^{-1}	g·day^{-1}	%·day^{-1}	kJ·day^{-1}
Estuary entry–exit	19.2 (3.3)	0.33 (0.10)	0.39 (0.12)	0.07 (0.03)	0.98 (0.47)	0.24 (0.11)
Estuary exit – summer	29.3 (8.1)	1.12 (0.30)	1.25 (0.15)	0.79 (0.17)	5.24 (0.68)	2.59 (0.80)
Summer–fall	65.6 (9.1)	1.19 (0.13)	0.78 (0.08)	1.47 (0.16)	2.34 (0.25)	3.96 (0.45)
Fall–winter	158 (12)	0.51 (0.29)	0.21 (0.12)	1.13 (0.74)	0.56 (0.33)	2.33 (1.56)

Note: Changes in size and energy content per day expressed as linear function within interval; changes in %·day^{-1} expressed as exponential function.

Fig. 3. Mean (*a*) fork length (FL), (*b*) weight, and (*c*) age (± standard error, SE) for juvenile Chinook salmon (*Oncorhynchus tshawytscha*) during estuarine and first ocean year phases of their life cycle between 1995 and 2005. Number below each mean is the number of fish analyzed. *N* for weight is same as for FL. Error bars that are not seen are smaller than the symbols.



By the end of February – early March, Chinook salmon were 316 ± 5 mm FL, weighed 451 ± 26 g, and were 402 ± 6 days old.

Condition factor, *K*, differed among locations or times across years ($P < 0.0001$) and among years at each location or time ($P < 0.0001$). *K* declined in juvenile Chinook salmon transiting the estuary but increased rapidly after ocean entry (Fig. 4). The greatest condition index, 1.43 ± 0.01, was found in fish collected during the fall. Condition declined again between fall and winter but remained well above values in the estuary.

Fig. 4. Mean condition factor (Fulton's *K*) (± standard error, SE) for juvenile Chinook salmon (*Oncorhynchus tshawytscha*) during estuarine and first ocean year phases of their life cycle between 1995 and 2005. Number below each mean is the number of fish analyzed.

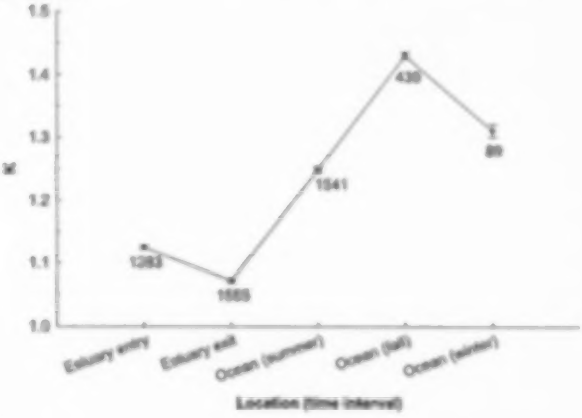
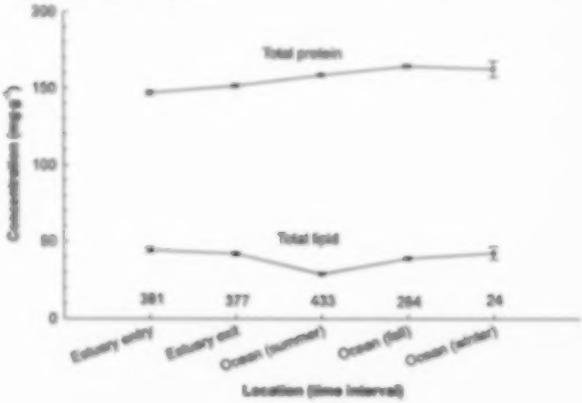


Fig. 5. Mean concentrations of total protein and total lipid (± standard error, SE) in juvenile Chinook salmon (*Oncorhynchus tshawytscha*) during estuarine and first ocean year phases of their life cycle between 1995 and 2005. Number below each mean is the number of fish analyzed.



Energy content

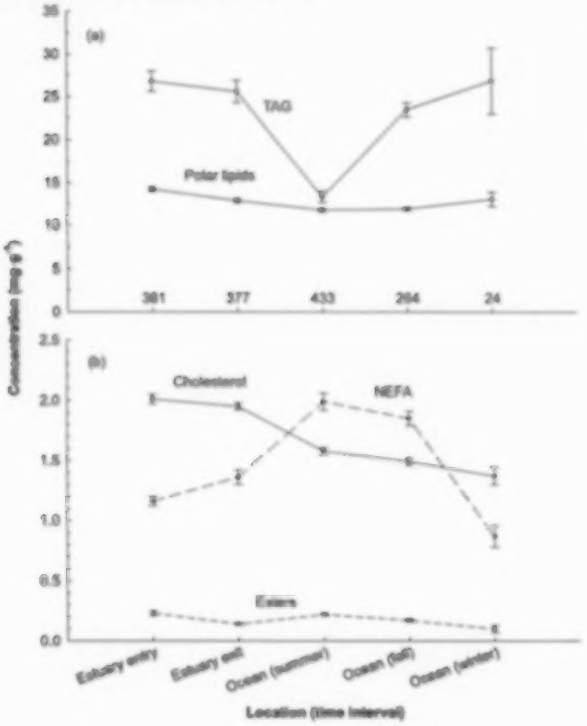
Total protein and total lipid dynamics during the estuarine and early ocean phase differed (Fig. 5). Whereas protein concentration increased significantly at each location or time between estuary entry and fall in the ocean ($P < 0.0001$), total lipid concentration declined between estuary

nile Chinook salmon (*Oncorhynchus tshawytscha*) while transiting the estuary and during the first ocean year.

	ΔLipids		ΔTAG		ΔTotal energy	
%·day^{-1}	kJ·day^{-1}	%·day^{-1}	kJ·day^{-1}	%·day^{-1}	kJ·day^{-1}	%·day^{-1}
1.20 (0.54)	0.03 (0.09)	0.12 (0.91)	0.02 (0.07)	–0.26 (1.42)	0.28 (0.18)	0.86 (0.59)
4.80 (0.93)	0.57 (0.22)	2.88 (0.81)	0.24 (0.15)	1.31 (0.98)	3.18 (0.89)	4.32 (0.87)
2.20 (0.29)	2.05 (0.33)	2.57 (0.27)	1.43 (0.30)	3.03 (0.36)	6.00 (0.63)	2.31 (0.27)
0.47 (0.31)	1.27 (0.63)	0.60 (0.34)	0.75 (0.22)	0.66 (0.34)	3.60 (2.19)	0.51 (0.31)

Standard error (SE) in parentheses; %·day^{-1} calculated from initial value at the start of the interval.

Fig. 6. Mean concentrations of lipid classes (± standard error, SE) for (*a*) triacylglycerols (TAG) and polar lipids, and (*b*) cholesterol, nonesterified fatty acids (NEFA), and steryl – wax esters in juvenile Chinook salmon (*Oncorhynchus tshawytscha*) during estuarine and first ocean year phases of their life cycle between 1995 and 2005. Numbers above the *x* axis in (*a*) are the numbers of fish analyzed for lipid classes in (*a*) and (*b*).



exit and the summer ($P < 0.05$) and then returned to levels similar to those in the estuary by the fall. Lipid concentration was preserved in the winter. The greatest mean total lipid concentration, 44.4 ± 1.3 mg·g^{-1}, was at the estuary entrance, but total protein peaked in the fall at 164.7 ± 1.2 mg·g^{-1}. Lipid and protein concentrations were significantly different among years at each location or time ($P < 0.0001$).

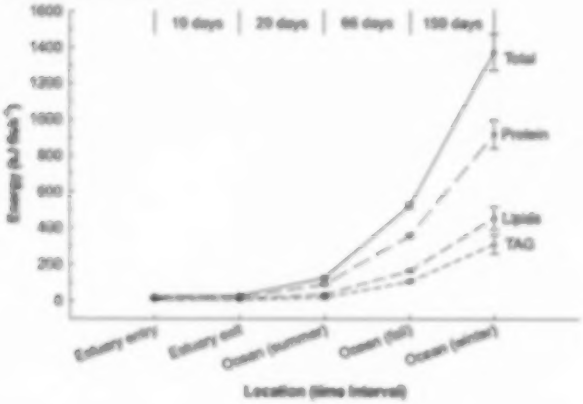
Almost the entire decline in total lipids during the 29 days between ocean entry and the summer sampling period was due to depletion of TAG (Fig. 6*a*). TAG concentrations were not significantly different among all locations or times except during the summer in the ocean, which was significantly different from all other periods ($P < 0.0001$). TAG concentrations declined 48% following ocean entry. There were significant differences in TAG concentrations among the 11 years ($P < 0.0001$).

Polar lipids, which quantitatively are mostly biomembrane phospholipids, varied across locations or times ($P < 0.0001$) (Fig. 6*a*). There was a steady loss of polar lipid concentration between estuary entry, estuary exit, and in the summer in the ocean ($P < 0.05$). Between summer and winter, polar lipid concentrations were statistically constant. Polar lipid concentrations varied significantly among years ($P < 0.0001$).

The other three lipid classes (cholesterol, NEFA, and esters) were found in much lower concentrations than TAG or polar lipids (Fig. 6*b*). Cholesterol, another primarily biomembrane component, declined between estuary entry and the winter ocean ($P < 0.0001$). The greatest depletion in concentration was between estuary exit and the summer, when growth rate was the greatest. As with polar lipids, this decline suggests that lower concentrations are due to lower structural lipid concentrations as body mass increases. NEFA dynamics reflect increased lipid mobilization and metabolism while in the estuary and ocean in the summer and fall (Fig. 6*b*). The highest levels co-occurred with the period of greatest growth in the summer and fall (Table 1). Steryl–wax esters varied among locations or times ($P < 0.0001$) but remained at low concentrations throughout the estuarine and early ocean periods (Fig. 6*b*). The highest concentrations were at estuary entrance and in the summer ocean. Esters did not contribute substantially to the energy budget of juvenile Chinook salmon.

The energy content of juvenile salmon is the sum of somatic tissue mass, mostly muscle protein, and stored fuels, primarily TAG. At estuary entry, juvenile Chinook salmon had 23.3 ± 0.8 kJ of total energy (Fig. 7). Approximately, 65% was protein and 35% was lipid, of which 61% was TAG. At estuary exit, however, total energy content of individuals was statistically unchanged from estuary entry. In the 29-day interval between ocean entry and capture in the summer, total energy content increased to 133.0 ± 6.7 kJ. Increasing growth rate following ocean entry resulted in protein comprising 72% of the total. Growth also contributed to a decline in TAG concentration, which in the summer was 52% of lipid energy content. By the fall, the average juvenile Chinook salmon contained 462.6 ± 14.2 kJ of energy, 69% of which was protein. The accumulation of TAG between summer and fall resulted in an increase in the proportion of total lipid that was TAG. In the fall, TAG was 63% of total lipid content. During the winter, after 159 more days, total energy content of a juvenile Chinook salmon was 991.7 ± 101.1 kJ. In winter, protein content declined to 62% of the total energy, and TAG was 65%, similar to the

Fig. 7. Mean energy content for TAG, total lipids, total protein, and total energy (± standard error, SE) in juvenile Chinook salmon (*Oncorhynchus tshawytscha*) during estuarine and first ocean year phases of their life cycle between 1995 and 2005. Scale at top denotes mean number of days that juvenile Chinook salmon spent in each location–time interval.



ratio in fall. All locations or times differed significantly ($P < 0.0001$) in energy content, except estuary entry and exit, which were not significantly different. There were statistically significant differences in energy content among years at all locations or times ($P < 0.0001$).

From the perspective of rate, the greatest accumulation of energy occurred between estuary exit and summer ocean sampling (29 days), the time of greatest growth rate in mass (Table 1). Total energy accumulation was 4.3%·day^{-1}, protein energy was about 5%·day^{-1}, and total lipid energy was 3%·day^{-1} in relation to energy content at the start of the interval. TAG increased at 1.3%·day^{-1} during the first 29 days at sea. The greatest daily increase in TAG, however, was between the summer and fall, when it accumulated at 3%·day^{-1}. During this same 66-day interval, protein accumulated at about 2%·day^{-1}, robust but less than during the first month at sea. The lowest daily percentage increase in total energy, 0.5%·day^{-1}, was in the winter in the ocean; estuarine energy accumulation was not significantly different. In the estuary, protein accumulation was 1%·day^{-1} and lipid content increased at 0.1%·day^{-1}, of which TAG declined by –0.3%·day^{-1} (Table 1). Thus, the greatest gain in protein (and somatic tissue) growth occurred not in the estuary, but soon after ocean entry. As the first ocean summer progressed, protein gain slowed and lipids, particularly the storage lipid TAG, became an increasing proportion of total energy accumulation. As might be expected, both protein and lipid accumulation declined substantially during the first winter at sea.

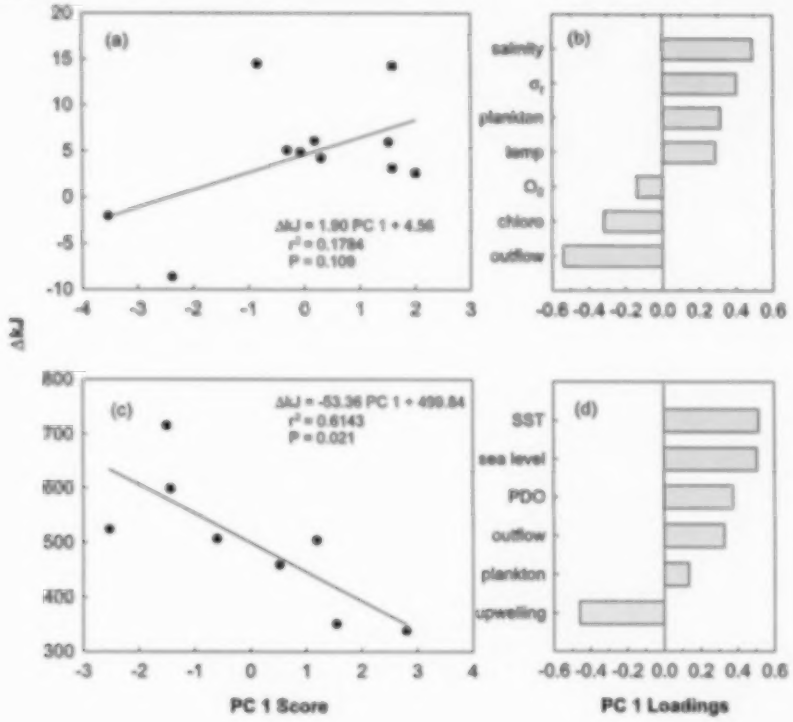
On a quantitative basis, the greatest daily energy accumulation was between summer and fall ($P < 0.0001$), when mass increased 246% in the 66-day interval. Juvenile salmon acquired 6.0 ± 0.6 kJ·day^{-1} during that period (Table 1). Daily, all components of the energy pool (protein, lipid, TAG) increased the most during the summer-to-fall period. The lowest rates of daily energy accumulation were during the estuarine phase of the juvenile Chinook salmon life cycle.

Table 2. Estimated consumption of northern anchovy (*Engraulis mordax*) equivalents for juvenile Chinook salmon (*Oncorhynchus tshawytscha*) in each location–time interval during estuarine and first ocean year life cycle phases.

			Juvenile Chinook salmon				Northern anchovy consumed	
Interval	No. of days	Temp. (°C)	Energy density (kJ·g^{-1})	Start weight (g)	End weight (g)	Body weight gained per day (%)	Grams per day[a]	No. per day[b]
Estuary	19	17.2	4.42	6.5	7.4	8.2	0.57	1.1
Ocean, entry–summer	29	12.6	4.55	7.4	39.1	15.3	3.56	3.2
Ocean, summer–fall	66	12.6	4.43	39.1	117	6.4	4.97	1.6
Ocean, fall–winter	159	12.6	4.27	117	452	4.1	11.8	3.7

Note: Modeled temperatures (Temp.) were mean of annual means for 1995–2005 where fish were sampled.
[a]Energy density of *E. mordax*, 3.11 kJ·g^{-1} wet weight, calculated from *E. encrasicolus* data (Tirelli et al. 2006).
[b]Number of *E. mordax* based on standard length of 40 mm (0.53 g) for estuary, 50 mm (1.1 g) in summer ocean, and 70 mm (3.2 g) in fall and winter ocean. Sizes of anchovies determined by standard lengths, rounded to nearest 5 mm, of anchovies in salmon stomachs (R.B. MacFarlane, unpublished data).

Fig. 8. Principal components analysis of relationship between first principal component (PC 1) of environmental factors and changes in energy content (ΔkJ) of juvenile Chinook salmon (*Oncorhynchus tshawytscha*) during (*a* and *b*) estuarine and (*c* and *d*) ocean entry to fall phases of their life cycle between 1995 and 2005. Each point is the mean change in energy content for each year. Loadings of environmental factors for each phase are in (*b*) for estuarine and (*d*) for ocean entry to fall. For estuarine influences, salinity, density (σ_t), zooplankton concentration, water temperature, oxygen concentration (O_2), chlorophyll *a* concentration, and freshwater outflow rate were evaluated. In the ocean, sea surface temperature (SST), sea level height, Pacific Decadal Oscillation (PDO), freshwater outflow, zooplankton concentration, and upwelling index were used.



All lipid and protein concentration and energy content variables differed among years and location–time interactions ($P < 0.0001$). The small standard errors of the means for all years combined are largely due to the relatively large sample size for each mean, but they also suggest that the variability of cruise dates did not compromise the validity of the combined data.

Prey consumption estimation

Using the Wisconsin bioenergetics model for Chinook salmon growth, it is possible to estimate the ration necessary to account for the size gains seen in each location–time interval. The results show that a small number of anchovies is required to account for the increases in energy content found (Table 2). In the estuary, consumption of about one northern anchovy per day (0.57 g·day^{-1}) would be adequate to account for the gain. In the ocean, consumption of about 13 northern anchovies every 2 days (4.97 g·day^{-1}) in the summer-to-fall interval would suffice, and three to four per day are required (11.8 g·day^{-1}) during the fall–winter period. During the rapid growth period following ocean entry, about three anchovies per day (3.56 g·day^{-1}) are needed to meet energy gains, representing about 15% of their body weight per day (based on mean body weight at midpoint of the interval).

Environmental influences on energy content

An objective of this study was to determine the relationship of environmental variables on energy content dynamics. To accomplish this, the mean annual total energy gain within the estuarine interval and between ocean entry and fall were assessed separately because hydrologic and oceanographic influences are likely to differ in importance between the two habitats. In the estuary, principal component one (PC 1) explained 18% of the variation in ΔkJ ($P = 0.11$) (Fig. 8*a*). PC 1 contained 43.5% of the interannual variability of environmental factors. Variables primarily driving this relationship were freshwater outflow and salinity (Fig. 8*b*). Apparently, lower outflow and higher salinity conditions are more favorable to energy gain. Outflow alone explained 36% of the energy change in the estuary by simple correlation analysis. PC 2 accounted for an additional 33% of the variance in environmental factors among years; lower temperature and higher dissolved oxygen were the variables most influencing a positive energy gain. Notably, plankton concentration, representative of prey abundance for juvenile Chinook salmon (MacFarlane and Norton 2002), contributed positively to energy accumulation but did not load as strongly as outflow and salinity.

From ocean entry to the fall, juvenile Chinook salmon energy content gain was significantly related to PC 1 ($P = 0.02$), explaining 61% of ΔkJ and composed mainly of sea surface temperature, sea level height, and upwelling (Figs. 8*c*, 8*d*). PC 1 explained 54.8% of the interannual variance of environmental factors. PC 2 was composed mostly of plankton concentration and freshwater outflow and explained another 27% of the variance. Conditions most favorable to juvenile Chinook salmon growth and energy gain are lower sea surface temperatures (positive loading on a negative relationship), lower sea level height (i.e., increasing southerly advection), and greater upwelling. By correlation analysis, sea surface temperature was related to ΔkJ with an $r^2 = 0.66$ ($P < 0.02$).

Discussion

The Chinook salmon assessed in this study came from the Central Valley of California. By limiting collection to April through June in the San Francisco Estuary, fall-run fish were targeted. Greater than 90% of the Chinook salmon from the Central Valley are fall-run fish (Pacific Fisheries Management Council (PFMC) 2006), which emigrate through the estuary in April to June (Fisher 1994). Because the only source of juveniles to the estuary is at the single entry from the Central Valley, fish aging at the entry and exit can estimate mean individual estuarine residence. In the coastal ocean between Pt. Arena and Cypress Pt., there are no other substantial sources of juvenile Chinook salmon (Myers et al. 1998; Good et al. 2005). The only other source is the Russian River, where fewer than 500 adults return yearly (Good et al. 2005), whereas the annual mean escapement of Chinook salmon to the Central Valley between 1995 and 2005 was 409 000 fish (PFMC 2006).

Growth and energy content

The results of the analyses presented here support the importance of the early ocean phase as a critical period for Chinook salmon (Mueter et al. 2005; Quinn et al. 2005). The greatest rate gains in size and energy content, relative to these values at the start of other location–time intervals, occurred during the first month in the ocean (1.3%·day^{-1} FL, 5.2%·day^{-1} weight, 4.3%·day^{-1} energy content), followed by the next two months. Rapid growth and energy storage confer greater survival potential (Pearcy 1992) to later ocean stages when other factors such as growth-related mortality may become important in establishing mature adult year-class strength (Beamish and Mahnken 2001). Of course, all data come from survivors at the time of capture, thus greater mortality of smaller individuals may influence mean size and growth determinations at later times, as is the case with all field collections. If, for example, mortality occurred in the smallest 50% of the population during the first month in the ocean (i.e., remove the smallest 50% of samples from ocean entry collection and then recalculate growth during the first month at sea), the apparent gain in weight would change from 5.2%·day^{-1} to 4.7%·day^{-1}. At 90% mortality, apparent weight gain would be 3.2%·day^{-1}. Thus, although lowering growth rate estimates, substantial mortality of smaller individuals still results in high growth rates for survivors during early ocean life. The gains seen in the first three months at sea were mostly due to protein added in the growth of somatic tissues, but substantial increases in total lipids and TAG also contributed to the increased rate of energy accumulation. Conversely, the lowest rates of growth and energy storage occurred during the first winter in the ocean and in the estuary. Low winter values were likely attributable to reduced forage biomass during this period of low ocean productivity (Smith 1968; Hickey 1989; Robinson et al. 1993).

Low estuarine growth and energy accumulation seen in this study may not reflect a ubiquitous situation. Data from other estuaries suggest that there may be greater growth in some estuaries. Whereas juvenile Chinook salmon in the Campbell River estuary (Korman et al. 1997), Duwamish River estuary (Salo 1969), and other estuaries on Vancouver Island and the Fraser River (Levy and Northcote 1982; Healey 1991) grew at rates similar to those found in the present study (0.21–0.62 mm·day^{-1}), in other estuaries, growth may be greater. Reimers' (1973) data suggest growth rates of 0.07 mm·day^{-1} during the summer and 0.9 mm·day^{-1} in the spring in the small estuary of the Sixes River in Oregon.

It is possible the nursery function for juvenile Chinook salmon may occur in an estuary or in the coastal ocean soon after entry. The present study strongly indicates that coastal waters are the primary site for rapid growth and energy gain. Estuarine growth was found to be 0.33 mm·day^{-1} but was 1.12–1.19 mm·day^{-1} during the first one to three months in the ocean. Juveniles in the Georgia Strait, captured shortly after estuarine residence, grew at 0.8–1.32 mm·day^{-1} (Healey 1980), and growth off the Oregon coast was 0.75–1.05 mm·day^{-1} (Fisher and Pearcy 1995), both consistent with the early ocean phase as being critical to rapid growth. The location of the nursery function may evolve according to relative prey availability and predation pressure of alterative locales. Although the freshwater delta portion of the San Francisco Estuary serves as a nursery for presmolt stages (i.e., <70 mm FL) (Kjelson et al. 1982), the saline portion does not. That may not have been the case historically, when a much greater area of brackish and salt marsh habitat existed, but there is no record of broad usage in the recent past (last 100 years). The Gulf of the Farallones is a highly productive, partially sheltered, coastal embayment that receives substantial inputs of nutrients from the estuary and a very high level of coastal upwelling (the highest along the West Coast of North America; NOAA Coastal Upwelling Indices, http://upwell.pfeg.noaa.gov/products/PFEL/modeled/indices/upwelling/NA/click_map.html), thus providing a plausible attraction to its evolution as a nursery for Central Valley salmonids. Also, by being a largely clupeiform-dominated fish community (Orsi et al. 2007), it provides abundant alternative prey to predators while allowing the relatively small outmigrating cohort of juvenile salmon to intermingle in clupeid shoals (J.A. Harding, A.J. Ammann, and R.B. MacFarlane, Southwest Fisheries Science Center, 110 Shaffer Road, Santa Cruz, CA 95060, USA, unpublished data), thus masking them from predator pursuit.

Further evidence of the importance of the early ocean phase can be found by changes in condition factor (*K*). Weight per unit length declined significantly while in the estuary. The lower *K* at estuary exit was not due primarily to

lower lipid concentrations, which did decline during estuary transit albeit to a small extent, but rather to the allometric increase in length (cubed) relative to weight. Greater length, relative to weight, may confer increased predator avoidance; longer fish swim faster. Also, smoltification, known to reduce condition, was not a factor; earlier study found that smoltification, measured as gill Na^+,K^+-ATPase activity, was complete before estuary entry (R.B. MacFarlane, unpublished data). Following ocean entry, K increased greatly through the fall during the time of abundant forage (Ainley 1990) and declined during the winter low-productivity period.

Despite declining condition through the estuarine phase of the fish's life cycle, protein concentration increased throughout the entire period from estuary entrance to ocean entry and on to fall in the ocean. Thus, as salmon grew, the proportion of their mass that was protein increased, which was at the expense of water concentration (R.B. MacFarlane, unpublished data). Protein concentrations are known to be resistant to environmental variability (Shearer et al. 1997; Edsall et al. 1999) or differences in locomotion (Alsop and Wood 1997).

Surprisingly, total lipid concentration was also conserved through the estuarine and summer–fall ocean period, except for a decline during the first month in the ocean. The data indicate that rapid growth in somatic tissues during this time required the catabolism of lipids, but as growth rate slowed later in the summer and into fall, there was no longer a trade-off in energy stores and somatic growth. This strategy would appear to position the species to accommodate the reduced forage conditions in the winter, yet allow enough growth in the summer to lower predation pressure. That the cause of the lower total lipid concentration during the summer was due entirely to the decline in TAG concentration, the primary form of metabolically available stored energy, and that TAG levels rebounded rapidly with somatic growth in the summer–fall supports these contentions. The increase in condition following ocean entry into the fall was the consequence of greatly increased somatic tissue (muscle protein) mass relative to gains in FL. Also, declining lipid, which has lower density than protein, contributed to the gain in fish body density.

The other lipid classes' dynamics revealed attributes of juvenile salmon development. Polar lipids and cholesterol concentrations, both primarily biomembrane constituents, declined commensurate with rapid growth during summer and fall in the ocean, suggesting that the cellular mass-to-membrane ratio increases with size. NEFA concentrations were elevated during the summer and fall, again consistent with the mobilization then catabolism of TAG to fuel the increase in anabolic somatic growth.

From a total energy perspective, including both somatic tissue and energy storage components, energy compartmentalization between protein and lipids was remarkably stable in juvenile Chinook salmon over the estuarine and first ocean year periods. Protein comprised 65%–72% of the total energy, lipid was 28%–35% of total energy, and TAG energy was 52%–69% of total lipid energy content over all sampling periods. Highest proportions of protein and lowest proportions of total lipids and TAG were during rapid growth in the first month in the ocean. These data show strong homeostatic regulation of body composition over an 11-year time span, regardless of variability in environmental conditions and forage abundance.

Prey consumption estimation

The quantity of food required to meet the increased size of juvenile Chinook salmon within each location–time interval was variable depending upon interval. In the estuary, the net gain in total body weight could be met by consuming the equivalent of one anchovy per day. During the summer-to-fall interval in the ocean, about one to two anchovies every day would be adequate, whereas three to four would suffice during the fall-to-winter period. These ration levels are greater than those reported by Benkwitt et al. (2009) for juvenile Chinook salmon off the coast of Oregon. They found that depending on the model used, juvenile Chinook salmon in the 83–122 mm FL range consumed 2%–4.3% body weight·day^{-1}. Their method was completely different than the one used here; they determined feeding rate by stomach fullness and laboratory-derived evacuation rates. My calculations, as a percentage of body weight per day for mean size fish at the midpoint within each interval, found that juvenile salmon needed to consume 8.2% body weight·day^{-1} in the estuary, 6.4% body weight·day^{-1} during the summer–fall interval in the ocean and 4.1% body weight·day^{-1} in the fall–winter period. During the rapid energy accumulation period from ocean entry to the summer sampling, covering 29 days on average, juvenile salmon needed to consume about three northern anchovies per day, or 15% body weight·day^{-1}.

Consumption rates found in the present study are not out of line with other determinations. Sagar and Glova (1988) found that juvenile Chinook salmon while in the river required 8.3% body weight·day^{-1}, similar to the 8.2% body weight·day^{-1} calculated for within-estuary consumption here. Juvenile chum salmon, *Oncorhynchus keta*, were estimated to consume from 6.3% to 10.5% body weight·day^{-1} during early ocean residence in Southeast Alaskan coastal waters (Orsi et al. 2004) and between 6.6% and 13.1% body weight·day^{-1} on the British Columbia coast (Godin 1981).

Reported consumption rates on the order of 2%–4%·day^{-1} would seem to constrain growth to quite low levels. Assuming increased somatic mass approximates 20%–30% of ingested food in young salmonids (Brett 1995), these consumption rates would allow gains in weight of only 0.4%–1.2%·day^{-1}, well below many found in the literature (Weatherly and Gill 1995) and in the present study.

For juvenile Chinook salmon, model input data on interval length and fish size were based on age determinations of fish measured during collections at the estuary exit in May and June and those sampled in the ocean off the estuary in May through August. Mean energy density values for juveniles from each location–time interval ranged from 4.27 kJ·g^{-1} to 4.55 kJ·g^{-1} and were midrange of those found by Trudel et al. (2005).

Environmental influences on energy content

There are scant data on the relationship between physical factors and salmon growth or energy content in the literature. It has been hypothesized that juvenile salmon, in particular ocean-type Chinook salmon, utilize lower-salinity waters in estuaries to ease the transition between freshwater and

saltwater (for example, see summary in Quinn (2005)), implying that lower salinity and greater freshwater inflow may be beneficial to juvenile salmon development. The data presented here would suggest that in larger estuaries such as San Francisco Estuary, this state might be subordinate. Quinn (2005) provided evidence that salmon in estuaries of different sizes may utilize them differently. In San Francisco Estuary, salinity and freshwater outflow appeared to have the greatest influence on the accumulation of somatic tissue and stored energy in juvenile Chinook salmon; however, years when energy content was high were years when the estuary was saltier and had lower freshwater discharge. The data suggest that the marine influence results in higher plankton concentrations, which may directly or indirectly (as prey for small fish) result in greater energy accumulation. In coastal waters off California and Oregon, subyearling Chinook salmon seem to prefer saltier waters as well (Fisher et al. 2007). Interestingly, lower flow conditions may also result in longer residence times within the estuary, but that was not found to be the case; there was no significant correlation between residence time and freshwater outflow ($R^2 = 0.02$, $n = 11$).

Ocean conditions conducive to high biological productivity in upwelling-dominated ecosystems (Barber and Smith 1981; Chavez 1996) were positively associated with greater growth and energy accumulation in juvenile Chinook salmon during the first summer and fall (approximately three months) in the coastal ocean. Lower sea surface temperatures, lower sea level height, and greater upwelling of subsurface waters were positive factors in energy gain and growth. Although not obligate, these variables are often related to one another. Strong northerly winds drive upwelling, which brings colder subsurface waters to the surface and also increases Ekman transport of coastal water offshore, thus depressing the sea level height. There are situations, however, in which upwelling is restricted to the upper mixed layer, and if that layer is deep and composed of warmer waters from southerly areas, the sea surface temperature remains warmer.

On an analytical note, for both habitats, one variable alone — freshwater outflow in the estuary and sea surface temperature in the ocean — explained as much or more of the interannual variability in ΔkJ as the multivariate principal component analysis. This is noteworthy and may suggest little need for multivariate evaluation, but I argue that this treatment has value because organisms and processes are rarely driven by one variable. By using PCA, a more integrated, inclusive assessment of factors shaping energy accumulation, or any other process, is obtained.

Other investigations have found relationships between ocean conditions and juvenile salmon survival, which may be related to growth and energy accumulation. Temperature is often found to be a factor in salmon ecology, but the direction of the relationship varies. Quinn et al. (2005) found an inverse relationship for both Chinook and coho salmon (*Oncorhynchus kisutch*), whereas farther north in the Bering Sea, warmer temperatures were beneficial to juvenile sockeye (Farley et al. 2007). Conditions such as El Niños that warm temperate coastal waters have been shown to reduce Chinook salmon survival and growth (Johnson 1988), but MacFarlane et al. (2005) showed that during the 1998 El Niño, juvenile Chinook salmon off central California had significantly greater growth, perhaps mediated by greater metabolic capacity and greater food supply. Cooler ocean temperatures were positively related to survival of Pacific salmon stocks off British Columbia and Washington, but warmer waters off Alaska were beneficial (Mueter et al. 2002).

The oceanographic variables sea surface temperature, sea level height, and upwelling, which related to growth and energy content of juvenile Chinook salmon, were the same variables that explained other dynamics in Chinook salmon. Botsford and Lawrence (2002) evaluated co-variability of these variables and the dynamics of Chinook and coho salmon and Dungeness crab in the California Current ecosystem from 1950 to 1990. They found that the species responded in different ways, but in general, cooler temperatures had a positive effect. Wells et al. (2008) found that these three variables affected growth of Chinook salmon, based on scale analysis of adult spawners from Alaska, Washington, and California, with fish south of the transition zone (region along the coast where the Subarctic Current bifurcates into the Alaska Current and the California Current, typically off Vancouver Island) responding well to the same conditions found in the analyses presented here. During the first year at sea, juvenile Chinook salmon originating from northern California grew better when spring and summer water temperatures were cooler, sea level height was lower, and upwelling was strong (Wells et al. 2007). From information in the literature and the results presented in this paper, there is a consistent pattern of oceanographic factors and their influence on juvenile Chinook salmon growth and accumulation of energy. Cooler sea temperatures, strong upwelling, and strong southerly advection in the California Current result in gains in size and energy content.

With the onset of changing estuarine and ocean conditions because of global climate change, the conditions that are beneficial for energy accumulation and growth may not be as prevalent in the future. There is concordance in climate change models that cold- and cool-water fish species, in particular salmonids, will likely be the most negatively impacted (Gallagher and Wood 2003; Intergovernmental Panel on Climate Change (IPCC) 2007). Warming and changing from the current hydrograph of winter rains and spring snowmelt to a situation dominated by more winter runoff and significantly less snowmelt will have profound effects on juvenile salmon emigration, growth, and survival in western mountains by the middle of the 21st century (IPCC 2007).

Climate change impacts will occur within estuaries. Although having received little attention to date from the climate-change research community, it can be expected that estuaries will become more saline with rising sea level and reduced freshwater outflow, which will in turn affect trophic resources (Harley et al. 2006), all three variables found in the present study to influence juvenile Chinook salmon energy accumulation while in the estuary. Further, changes in the timing of freshwater runoff, often predicted in climate-change models (IPCC 2007), may alter temporal patterns of estuarine salinity and marine-influenced plankton distributions and become decoupled from juvenile salmon migratory behavior, thus adversely impacting growth and energy dynamics.

According to the Intergovernmental Panel on Climate Change, future climate impacts will have greater effects on coastal species such as Chinook salmon than on more oceanic species (IPCC 2007). Although models relating to spatial and temporal variability of climate change on marine ecosystems need improvement and are not consistent on the direction and intensity of ocean responses, some predict decreases in upwelling and the formation of a more stratified, deeper-mixed layer that adds to the increase in sea surface temperature and reductions in biological productivity (Cox et al. 2000; Loukos et al. 2003; Sarmiento et al. 2004). Others predict no real change in intensity or seasonality over the next century (Mote and Mantua 2002), while still others predict an intensification of coastal upwelling in the California Current system, but coupled with higher sea surface temperatures (Schwing and Mendelssohn 1997), or delayed until later in the year (onset changes from April to July) (Snyder et al. 2003). Because upwelling and lower temperatures were found to be the primary determinants of greater energy accumulation and increase in somatic tissue in juvenile Chinook salmon in the present study, changes in upwelling and associated physical properties would have a pronounced effect on salmon growth and energy dynamics during the first summer at sea. Reduced or delayed upwelling would result in lower growth and energy accumulation rates at the time of ocean entry and would likely result in reduced survival. Increased upwelling, by contrast, may improve growth and subsequent survival, but if upwelling is too strong, offshore advection of biological productivity (Connolly and Roughgarden 1999) may remove food resources from the inshore habitats utilized by juvenile Chinook salmon and be of little benefit.

The results of this 11-year study of energy content and growth of juvenile Chinook salmon provide a baseline in space and time to compare against future estuarine and ocean conditions. The time period of the data gathering, 1995–2005, spans several important oceanographic intervals, including a regime shift, El Niño, and La Niña, and thus represent the current mean profile of energy accumulation of juvenile Chinook salmon during the estuarine and first ocean year phases of their life cycle. The data reveal that the greatest rates of somatic tissue growth and energy accumulation occur during the first 30 days or so in the ocean. As the first ocean summer progressed, protein (somatic tissue) growth slowed as stored lipids, in the form of TAG, increased. This study found little evidence of significant growth or energy storage while the Chinook salmon were in the estuary, but this may be a more variable and system-specific situation. Other studies suggest that in some systems, estuaries provide an important nursery function (Reimers 1973; Healey 1982), but it appears that this function can be deferred until ocean entry in other cases. Thus, the original hypothesis of rapid growth during the estuarine phase is rejected, but the increasing importance of acquiring storage energy during the summer for subsequent winter survival is accepted.

The stated hypothesis that upwelling intensity and the related oceanographic variable sea surface temperature strongly influence energy accumulation in juvenile salmon, as previously implied (Botsford and Lawrence 2002; Wells et al. 2007, 2008), was supported by the results of the present 11-year study. Although plankton biomass was not the primary factor relating to energy accumulation in the estuary as hypothesized, higher salinity and lower freshwater outflow suggest that marine influences have a positive effect on energy accumulation, which may indicate that marine-derived prey may be beneficial. With impending changes in ocean and estuarine environments due to climate change, juvenile salmon energy dynamics and growth may change in response to environmental changes, or they may adapt to the new environment. In the lower part of the Chinook salmon range, which includes San Francisco Estuary, if projections of changes in the timing and decreases in quantity of freshwater runoff are realized (IPCC 2007), there may be beneficial, or at least not strongly adverse, consequences to juvenile Chinook salmon. Although models do not agree on the impacts of climate change to West Coast nearshore habitats, if coastal upwelling is delayed to later in the year or reduced in intensity, there will almost certainly be adverse effects to juvenile Chinook salmon growth and energy content, which will likely reduce survival. The data presented here offer a basis for comparison with future estuary and ocean conditions.

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Appendix A

Table A1. Annual means (± standard error, SE) at each location–time sampling.

	Estuary		Ocean		
Year	Entrance	Exit	Summer	Fall	Winter
Fork length (mm)					
1995	82 (1)	85 (1)	118 (7)		
1996	82 (0)	84 (2)	106 (3)		
1997	83 (1)	89 (1)	127 (10)		286
1998	88 (1)	88 (0)	111 (2)	188 (2)	305 (5)
1999	86 (1)	93 (1)	177 (3)	191 (5)	
2000	81 (1)	91 (1)	103 (2)	214 (6)	
2001	79 (1)	83 (1)	154 (6)	215 (5)	344 (9)
2002	82 (1)	85 (1)	111 (4)	211 (2)	247 (22)[a]
2003	80 (1)	86 (1)	156 (3)	199 (4)	
2004	82 (1)	89 (1)	149 (1)	193 (2)	
2005	79 (1)	85 (1)	110 (1)	202 (2)	
Weight (g)					
1995	6.5 (0.2)	6.6 (0.2)	27.2 (5.1)		
1996	6.4 (0.1)	5.9 (0.4)	16.1 (1.5)		
1997	6.4 (0.2)	7.2 (0.3)	49.3 (11.7)		333
1998	8.4 (0.4)	7.1 (0.1)	17.8 (0.5)	101 (4)	376 (20)
1999	6.9 (0.2)	8.9 (0.3)	83.0 (4.3)	93.7 (10.5)	
2000	5.8 (0.2)	7.8 (0.2)	16.0 (1.4)	147 (13)	
2001	5.4 (0.1)	6.6 (0.2)	61.4 (6.2)	145 (10)	612 (56)
2002	6.3 (0.2)	7.2 (0.2)	26.0 (3.7)	141 (5)	207 (62)
2003	6.1 (0.2)	7.4 (0.2)	60.8 (4.2)	119 (4)	
2004	6.4 (0.2)	8.3 (0.3)	45.2 (0.7)	106 (4)	
2005	5.7 (0.2)	7.0 (0.3)	18.5 (0.9)	124 (4)	
Age (days)					
1995	134 (2)	162 (2)	174 (4)		
1996	134 (2)	158 (3)	168 (3)		

Table A1 (*concluded*).

	Estuary		Ocean		
Year	Entrance	Exit	Summer	Fall	Winter
1997	136 (2)	176 (2)*	209 (17)		388
1998	134 (3)	146 (1)	160 (10)	256 (3)	403 (8)
1999	140 (3)	163 (3)	200 (7)		
2000	129 (3)	141 (4)	150 (5)	215 (40)*	
2001	126 (5)	142 (3)	182 (7)	246 (9)	
2002	119 (4)	124 (3)*	173 (9)	239 (9)	409
2003	130 (4)	136 (3)	191 (3)	225 (1)	
2004	131 (2)	154 (3)	199 (3)	244 (11)	
2005	122 (3)	148 (4)	163 (5)	239 (12)	
Protein (mg·g^{-1})					
1995	150 (2)	151 (2)	145 (3)		
1996	123 (5)	154 (6)	161 (4)		
1997	155 (4)	153 (4)	151 (6)		
1998	128 (4)	135 (3)	133 (5)*	166 (4)	165 (6)
1999	145 (3)	151 (3)	150 (4)		
2000	150 (2)	153 (2)	154 (2)	179 (1)	
2001	149 (2)	154 (2)	169 (2)	191 (2)*	
2002					155 (16)
2003	166 (4)	167 (3)	158 (3)	152 (3)	
2004	133 (3)	137 (2)	154 (2)	154 (2)	
2005	164 (3)	166 (3)	166 (2)	166 (2)	
Lipid (mg·g^{-1})					
1995	20.9 (2.5)	26.0 (1.2)	11.3 (0.5)*		
1996	39.5 (4.4)	36.0 (5.3)	22.6 (2.3)		
1997	29.7 (2.8)	32.9 (2.9)	17.6 (1.6)		
1998	47.5 (2.8)	33.7 (2.1)	25.8 (2.8)	28.7 (1.9)	34.9 (4.7)
1999	47.8 (4.5)	53.7 (3.7)	19.0 (1.6)		
2000	34.8 (2.7)	28.3 (2.0)	23.8 (1.6)	25.7 (1.2)	
2001	41.8 (2.4)	32.0 (2.5)	42.2 (2.5)	53.4 (3.7)	56.9 (3.8)
2002	58.5 (4.9)	52.8 (4.9)	54.6 (4.7)*	46.1 (3.4)	35.2 (16.6)
2003	43.8 (2.8)	42.2 (3.4)	39.2 (2.2)	52.7 (4.3)	
2004	60.8 (5.9)	65.8 (5.6)	21.0 (0.7)	38.6 (1.3)	
2005	40.4 (2.5)	36.3 (3.8)	25.6 (1.0)	35.2 (1.0)	
Total energy (kJ·fish^{-1})					
1995	20.8 (1.7)	18.7 (1.3)	37.6 (3.3)		
1996	14.9 (1.5)	19.1 (2.0)	52.6 (8.0)		
1997	20.4 (1.4)	26.4 (1.9)	24.2 (1.6)		
1998	29.6 (4.3)	21.0 (1.4)	50.2 (3.0)	360 (38)	1211 (128)
1999	27.5 (3.6)	32.4 (2.8)	210 (21)		
2000	22.6 (1.4)	25.8 (1.7)	58.7 (7.9)	533 (51)	
2001	20.5 (1.0)	23.1 (2.1)	265 (31)	739 (66)*	
2002					841 (459)
2003	25.8 (1.8)	30.9 (2.8)	242 (32)	491 (35)	
2004	26.9 (2.2)	41.2 (3.8)	164 (9)	393 (15)	
2005	24.0 (2.5)	38.5 (5.3)	77.7 (6.2)	542 (21)	

Note: Means are unadjusted for variation in age for each collection.

*Denotes annual mean is ±1 standard deviation (SD) beyond overall mean for variable in that location or time.

Bioenergetic responses of a benthic forage fish (*Fundulus heteroclitus*) to habitat degradation and altered prey community in polluted salt marshes

Daisuke Goto and William G. Wallace

Abstract: In this study, we examined bioenergetic responses of age-2+ and age-3+ mummichogs (*Fundulus heteroclitus*) to degraded habitat condition and impoverished benthic prey community in polluted salt marshes by incorporating in situ food consumption rates with the mercury mass balance model technique. In general, bioenergetic responses of mummichogs appeared to be more related to benthic prey community than habitat condition. Fish from prey-impoverished marsh (PIM) sites generally had significantly elevated total metabolic costs compared with those from non-prey-impoverished marsh (NPIM) sites. However, the PIM populations also had ~two- to three-fold higher consumption rates than the NPIM populations, suggesting that the PIM populations may be capable of adjusting food consumption to offset elevated metabolic costs. Furthermore, only age-2+ females of the PIM populations had significantly reduced growth rates, whereas there was little among-site differences for age-2+ males and age-3+ fish, indicating that an increased consumption by the PIM populations may also have been a compensatory mechanism to maintain growth. Most of the PIM populations, however, had substantially (up to 80%) lower growth conversion efficiency relative to the NPIM populations. These findings suggest that pollution-tolerant fishes such as mummichogs may have energetic costs of living in chronically degraded habitats.

Résumé : Dans notre étude, nous examinons les réponses bioénergétiques des choquemorts (*Fundulus heteroclitus*) d'âges 2+ et 3+ à des conditions d'habitat dégradées et à une communauté de proies appauvrie dans des marais salés pollués, en incorporant les taux de consommation de nourriture in situ par la méthode du bilan massique du mercure. En général, les réponses bioénergétiques du choquemort semblent être déterminées plus par la communauté benthique de proies que par les conditions de l'habitat. Les poissons provenant des sites de marais appauvris en proies (PIM) ont des coûts métaboliques totaux significativement plus élevés, comparativement aux poissons des sites non appauvris en proies (NPIM). Cependant, les populations des PIM ont aussi des taux de consommation ~deux à trois fois plus élevés que les populations des NPIM, ce qui laisse croire que les populations des PIM sont capables d'ajuster leur consommation de nourriture pour compenser les coûts métaboliques plus élevés. De plus, seules les femelles d'âge 2+ des populations des PIM ont des taux de croissance significativement réduits, alors qu'il y a peu de différences entre les sites en ce qui concernes les mâles d'âge 2+ et les poissons d'âge 3+; cela indique que la consommation accrue des populations des PIM a pu servir de mécanisme compensatoire pour maintenir la croissance. La plupart des populations des PIM, cependant, possèdent une efficacité de conversion de croissance considérablement réduite (jusqu'à 80 %) par rapport aux populations des NPIM. Nos résultats indiquent que les poissons tolérants à la pollution, tels que les choquemorts, encourent des coûts énergétiques pour vivre dans des habitats chroniquement dégradés.

[Traduit par la Rédaction]

Introduction

Trophic connectivity of salt marsh ecological landscapes is an important driver for ecosystem functioning in coastal environments (Levin et al. 2001). This connectivity may, however, be disrupted in urban coastal ecosystems due to degradation of habitat quality (e.g., chemical pollution and eutrophication; Able et al. 1998; Levin et al. 2001). Altered physicochemical properties of urbanized habitats can result in a considerable shift in biological assemblages (Warwick 2001). Benthic organisms are particularly vulnerable to these

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alterations in the environment, and shifts in benthic assemblages may directly or indirectly affect trophic links (e.g., Hinz et al. 2005; Powers et al. 2005), as well as trophically mediated processes including energy flow in coastal ecosystems (Levin et al. 2001). Because sediment-associated organisms such as benthic invertebrates can have species-specific differential tolerance to anthropogenic stressors, degradation of water quality in urban salt marshes subsequently may alter the availability of prey resources to benthivorous fishes (Boesch 1982).

Altered trophic ecology is often intimately associated with energetic performances (e.g., elevated metabolism) of fish (Rose 2000; Eby et al. 2005). There is, however, a limited understanding of the underlying mechanisms of how altered habitat condition and food web structure influence energetic responses of benthic fish in urban coastal habitats (Livingston 1984; Rose 2000). Furthermore, anthropogenic stressors can often cascade through food chains (Clements and Rees 1997; Farag et al. 1999), resulting in a variety of adverse effects in fish (Hammerschmidt et al. 2002; Farag et al. 2003), including disruption in energetic balance (Sherwood et al. 2000; Campbell et al. 2003). Chronic exposure to pollutants through the diet can, for instance, induce physiological stresses and increase metabolic activities (e.g., sequestration and detoxification of chemicals) in fish (Handy et al. 1999; Campbell et al. 2002).

Fishes living in chronically degraded habitats may also compensate for additional energetic costs incurred by anthropogenic stressors (e.g., pollutants; Levin et al. 2001; Campbell et al. 2002). Because energy allocation to metabolism precedes that to growth and (or) reproduction, stress-induced metabolic activities can indirectly impair growth and reproduction of fish (Health 1995; Brown et al. 2003). Wild populations of fishes chronically exposed to anthropogenic stressors often exhibit altered life history traits such as lower growth rate, shorter life span, and fewer spawning occasions (Toppin et al. 1987; Moiseenko 2002). Furthermore, a re-allocation of energy to additional metabolic costs in fish may also reduce energy reserves for energy-demanding activities (i.e., metabolic scope for activity) such as foraging and predator avoidance (Handy et al. 1999; Campbell et al. 2002; Rennie et al. 2005). These alterations in energy allocation may ultimately reduce the fitness of fish and potentially lead to mortality (Campbell et al. 2002).

A bioenergetics modeling approach has been frequently used in fisheries research to examine the effects of biological and environmental factors on energetic performances of fishes and shellfishes (e.g., Grant 1996). As the effects of anthropogenic stressors on fish can be expressed by alterations in energy intake (i.e., food consumption) and expenditures (e.g., metabolism), bioenergetics modeling is a valuable tool for understanding their functional responses to alterations in habitat quality (Sherwood et al. 2000; Beyers and Rice 2002). Although there have been attempts to investigate habitat degradation impacts on fish bioenergetics, food consumption rates in many of these studies are laboratory-based or model-simulated (e.g., Muller and Nisbet 1997; Beyers et al. 1999*b*). Furthermore, a traditional approach to estimating in situ food consumption rates using the gut evacuation method is highly labor-intensive (Sherwood et al. 2002) and thus may be impractical for field-based comparative studies. Energetic responses of fish to habitat condition (e.g., stress-induced energetic costs) can, however, be difficult to detect or even misleading without in situ food consumption estimates (i.e., energy gain) (Sherwood et al. 2002; Morinville and Rasmussen 2003). Due to the logistical difficulties in estimating in situ food consumption rates of wild populations of fishes (Chipps and Wahl 2008), except for a few studies (e.g., Sherwood et al. 2000; Trudel et al. 2000), environmental impacts on food consumption and subsequent allocation of consumed energy have not been fully explored in the field setting (Beyers and Rice 2002).

The main objective of the present study was to elucidate how a pollution-tolerant fish, the mummichog (*Fundulus heteroclitus*; Pisces: Cyprinodontidae), thrives in chronically polluted tidal salt marshes in New York, USA, by examining their bioenergetics (food consumption, total metabolism, and growth conversion efficiency) with the mercury mass balance model technique (Trudel et al. 2000). Mummichogs are one of the most abundant and productive resident fishes in tidal salt marshes of the Northwest Atlantic coast (Meredith and Lotrich 1979). Because resident fishes are conduits of marsh production transport (Kneib 1997), alterations in bioenergetics of resident fishes can have further implications for energy cycling and productivity within coastal ecosystems (Kneib 2003).

Because of their relatively high tolerance to a variety of anthropogenic stressors, mummichogs can thrive in severely polluted coastal habitats (Weis 2002; Wirgin and Waldman 2004). Anthropogenic stressors such as chemical pollutants can, however, induce negative effects on foraging behaviors of mummichogs in these polluted habitats (Weis et al. 2001). In addition to altered prey availability (e.g., local extinction of sensitive benthic invertebrate species), these altered behaviors are likely to disrupt energy allocation processes of mummichogs in urban tidal salt marshes. In the present study, therefore, we hypothesized that mummichog populations living in the environment with elevated pollutants and impoverished prey community have (*i*) reduced food consumption rates, (*ii*) increased metabolic costs, and (*iii*) reduced growth conversion efficiency.

Materials and methods

Study area

The study was conducted in the Arthur Kill (AK; part of the New York – New Jersey (NY-NJ) Harbor Estuary Complex), which is a tidal waterway located between northern New Jersey and Staten Island, New York, connecting Newark Bay and Kill van Kull to the north and Raritan Bay, New York Harbor, and the Atlantic Ocean to the south (Fig. 1). Due to the proximity to industrial facilities, petroleum refining facilities, heavy shipping traffic, and sewage treatment plants, AK is one of the most severely polluted areas in the NY–NJ Harbor Estuary system (Steinberg et al. 2004). As a result of chronic pollution, the water quality of the NY–NJ Harbor Estuary has been severely degraded for more than a century (Crawford et al. 1994). Many fishes and shellfishes in the NY–NJ Harbor Estuary are directly or indirectly exposed to a variety of anthropogenic stressors, resulting in a considerable loss of biodiversity in this region over the years (Crawford et al. 1994). Among the anthropo-

Fig. 1. Map of study sites in the Arthur Kill (Mill Creek, Richmond Creek, Main Creek, and Neck Creek) and Raritan Bay (Lemon Creek), New York, USA.

genic stressors, chemical pollutants released with industrial and municipal discharges have been major contributors to the deterioration of trophic structure and ecosystem function in this region (Adams and Benyi 2003). The highest levels of some of these pollutants occur in AK tributaries (Weis et al. 2001). Because tides enter from both ends of AK, this region is poorly flushed, resulting in enhanced entrapment and accumulation of pollutants in sediments (Adams and Benyi 2003; Bopp et al. 2006).

Despite recent improvements in water quality of the NY–NJ Estuary Complex including AK (Bopp et al. 2006), the residual impacts of historic pollution (e.g., altered biological assemblages) can still be observed at many areas in the NY–NJ Harbor Estuary (Adams and Benyi 2003). Because the NY–NJ Harbor Estuary provides essential habitats for resident and migrant aquatic, as well as terrestrial, species (Able et al. 1998; Steinberg et al. 2004), benthivorous predators are likely to be indirectly affected by anthropogenic stressors. Various areas in the NY–NJ Harbor Estuary are the subject of current and future restoration (Bain et al. 2007), and thus it is critical to understand the effects of altered environmental condition and trophic dynamics in benthic habitats on energy flow in evaluating the success of remediation (e.g., coastal fisheries productivity).

Field sampling

Sampling of mummichogs and their macroinvertebrate prey was conducted twice per month during the summer (June to September) of 2004 at subtidal creeks of four AK tributaries (Mill Creek, Richmond Creek, Main Creek, and Neck Creek) and at a regional reference site near Raritan Bay (Lemon Creek) (Fig. 1; Tables 1 and 2). Samples were then pooled at the end of the sampling period for each site.

Mill Creek is located near the confluence of AK and Raritan Bay and has been polluted from historic smelting activities. Richmond Creek and Main Creek are tributaries of the Fresh Kills Complex. Fresh Kills Complex is one of the largest salt marshes in New York State; these creeks are surrounded by Fresh Kills Landfill, the largest landfill in the United States. Neck Creek is polluted with various chemicals because of its proximity to many oil refineries, as well as abandoned industrial facilities. Lemon Creek is one of a few relatively undisturbed tidal salt marshes in the region. Unlike the AK sites where there are only a large number of mummichogs and a small number of other forage fishes such as Atlantic silversides (*Menidia menidia*) and striped killifish (*Fundulus majalis*), at Lemon Creek, there are a number of nekton species, including blue crab (*Callinectus sapidus*), summer flounder (*Paralichthys dentatus*), bluefish (*Pomatomus saltatrix*), American shad (*Alosa sapidissima*), sheepshead minnows (*Cyprinodon variegatus*), and striped killifish (D. Goto, personal observation).

Adult mummichogs were collected using a 3.05 × 1.22 m seine net with 6 mm polyester mesh or unbaited cylindrical minnow traps with 6 mm mesh. The sampling continued until a representative size of the fish population at each site (n = 100 to 200) was captured. Captured fish were immediately placed on ice and subsequently transported to the laboratory. Sediment-dwelling macroinvertebrate prey were collected by using a 15 × 15 cm quadrat and sieving bulk surficial sediments through 500 μm mesh and transferred to an acid-washed glass jar. Epibenthic macroinvertebrate prey (e.g., *Palaemonetes* spp.) were collected using a D-frame dip net. Both sediment-dwelling and epibenthic macroinvertebrates were then transported on ice (<60 min) to the laboratory (note that placing macroinvertebrates on ice during transport did not lead to mortality).

Laboratory sample processing

In the laboratory, sediment-dwelling macroinvertebrates were sorted from detritus and identified under a stereomicroscope (4× or 16×) using species keys from the literature (Fauchald 1977; Weiss 1995; Merritt and Cummins 1996; Pollock 1998). Then, to minimize measurement errors associated with unassimilated mercury in the gut contents, both mummichogs and macroinvertebrates were allowed to depurate gut contents for at least 24 h in filtered 15‰ seawater. Mummichogs were then euthanized by an overdose of MS-222 (tricaine methanesulfonate), and each fish was sexed, measured (total length ± 0.01 mm), and weighed (wet weight ± 0.01 g). For age determination, approximately 10–20 scale samples were taken from each fish at the area behind the operculum and above the lateral line (DeVries and Frie 1996). Macroinvertebrates and mummichogs were then stored in a freezer (–80 °C) for mercury analyses.

Table 1. Physicochemical characteristics of Lemon Creek, Mill Creek, Richmond Creek, Main Creek, and Neck Creek during the summer (June to September).

	Study sites				
Environmental variables*	Lemon Creek	Mill Creek	Richmond Creek	Main Creek	Neck Creek
Temperature (°C)	25.2	28.3	29.6	26.0	31.6
Dissolved oxygen ($mg \cdot L^{-1}$)	6.13	6.53	3.07	2.80	16.1
Salinity (‰)	6.3	14.7	5.0	6.0	14.0
pH	7.68	7.26	7.88	7.93	8.70
Fine sediment particles (%)	33.5	74.7	63.8	17.5	59.5
Total organic carbon (%)	5.95	4.92	2.38	5.43	12.5
Trace metals in sediments ($\mu g \cdot g^{-1}$)					
Ag	0.83	1.38	0.78	1.59	1.37
Cd	1.20	2.88	1.58	2.90	1.08
Cu	125	912	99.7	216	153
Ni	27.9	36.5	34.0	70.3	49.0
Pb	83.9	656	91.9	171	215
Zn	220	1190	289	411	339

*Source of the data: Goto (2009).

Table 2. Benthic macroinfaunal assemblages (in percent biomass) at Lemon Creek, Mill Creek, Richmond Creek, Main Creek, and Neck Creek.

	Biomass by major taxonomic groups (%)*						
Study site	Polychaeta	Oligochaeta	Crustacea	Insecta	Gastropoda	Bivalvia	Total biomass (g dry weight·m^{-2})
Lemon Creek	67.24	1.04	0.94	0.37	16.02	14.39	7.77
Mill Creek	20.18	3.02	0.42	0.00	74.42	1.95	17.22
Richmond Creek	91.12	4.51	4.33	0.01	0.00	0.02	21.60
Main Creek	90.49	2.31	7.10	0.10	0.00	0.00	26.43
Neck Creek	92.92	3.62	2.45	0.01	0.03	0.32	9.64

*Source of the data: Goto and Wallace (2009b).

Food consumption rate estimation

Overview of the mercury (Hg) mass-balance model

Annual average daily food consumption rates of mummichogs were estimated using the Hg mass balance model technique (Trudel et al. 2000) for each individual fish on a daily basis (Tucker and Rasmussen 1999). Mercury is one of the most globally widespread trace elements and readily accumulates in fish. In this mass balance model technique, Hg is used as a chemical tracer to estimate in situ food consumption rates of fish in the field (Trudel et al. 2000). The majority of Hg accumulated in many fishes is predominantly taken up through dietary sources (Hall et al. 1997; Wang and Wong 2003), whereas the uptake of dissolved Hg or sediment-associated Hg is negligible in the natural environment (Weis et al. 1986; Trudel and Rasmussen 2006). Therefore, the trophic cycling of Hg can be used to indirectly estimate food consumption rates of fish by quantifying growth rates, daily Hg turnover rates in fish (Hg assimilation and elimination rates), and Hg body burdens in field-collected prey organisms and fish (Trudel et al. 2000). In the current study, mass-specific daily food consumption rates (C, $g \cdot g^{-1} \cdot day^{-1}$) of mummichogs were estimated using the following Hg mass-balance model, interpolating fish body size (g wet weight) and Hg whole-body burden in mummichogs ($ng \cdot g^{-1}$) between adjacent age classes (Trudel et al. 2000):

$$(1)\quad C = \frac{Hg_{t+\Delta t} - Hg_t \cdot e^{-(E+G+Hg_g)\cdot\Delta t}}{AE \cdot Hg_d \cdot \left[1 - e^{-(E+G+Hg_g)\cdot\Delta t}\right]}(E + G + Hg_g)$$

where $Hg_{t+\Delta t}$ and Hg_t are Hg body burdens in mummichogs ($ng \cdot g^{-1}$) at time $t + \Delta t$ and t (days), respectively, Hg_d is Hg body burdens in prey ($ng \cdot g^{-1}$), AE is Hg assimilation efficiency from prey, E is Hg elimination rate ($ng \cdot g^{-1} \cdot day^{-1}$), Hg_g is Hg in gonads released at spawning ($ng \cdot g^{-1} \cdot day^{-1}$), and G is specific growth rate ($g \cdot g^{-1} \cdot day^{-1}$).

Parameterization of the Hg mass-balance model — analyses of Hg in mummichogs

Because the majority of Hg accumulated in fish (including mummichogs; Weis 1984) is in the form of organic Hg (mainly methylmercury; Bloom 1992), total Hg content in fish was analyzed. Previously frozen fish samples were thawed at room temperature and then homogenized with a Polytron tissue homogenizer (Kinematica, Switzerland) in NANOpure (reagent-grade) water. Homogenized samples were oven-dried (~60 °C) for 24 to 48 h. Subsamples (~0.5 g dry weight) were weighed and transferred into an acid-washed 60 mL BOD bottle. Samples were then digested in a mixture (1:4 by volume) of concentrated trace-metal-grade nitric and sulfuric acids (10 mL) in a water bath (60 °C) for ~3 h and cooled in a refrigerator. Samples were oxidized with 5% potassium permanganate (10 mL) and 5% potassium persulfate (5 mL) at room temperature

overnight. The digested samples were transferred into 100 mL volumetric flasks, and 12% sodium chloride – hydroxylamine sulfate was added to the samples to reduce excess potassium permanganate. Samples were then brought to final volume with NANOpure water, filtered (0.45 μm), and refrigerated (for <3 days) or analyzed immediately with a cold vapor atomic absorption spectrometer (Perkin Elmer Flow Injection Mercury System, FIMS 100, USA) using stannous chloride as a reducing reagent.

Population-specific relationships between fish body size (g, wet weight) and total Hg whole-body burden were then modeled using nonlinear regression, which was fitted by the Levenberg–Marquardt least squares algorithm. The total Hg whole-body burden for back-calculated weight-at-age (as described below in Age determination and growth rate estimation) was first estimated for each individual fish (Appendix A, Table A1). Food consumption rates of individual fish were then estimated by interpolating back-calculated fish weight and Hg whole-body burden between ages of each individual on a daily basis (Tucker and Rasmussen 1999).

Analyses of organic Hg in prey

As organic Hg content in aquatic invertebrates is often lower than total Hg content (Malley et al. 1996), organic Hg was first extracted from macroinvertebrates by acid-leaching with methylene chloride and hexane (Malley et al. 1996). Previously frozen samples were thawed in an acid-washed 50 mL glass centrifuge tube, homogenized in NANOpure water, and then oven-dried (~60 °C) for 24 to 48 h. Dried samples were weighed and homogenized in a mixture of 30% sodium bromide (in 4 mol·L^{-1} sulfuric acid, 5 mL) and 2.5% copper sulfate (in 4 mol·L^{-1} sulfuric acid, 7.5 mL) for 1 min (Wagemann et al. 1997). Then, a mixture (3:2 by volume) of methylene chloride and hexane (10 mL) was added to the sample homogenates (Wagemann et al. 1997). Samples were vigorously shaken on an orbital shaker for at least 12 h and then allowed to stand until the samples settled. An aliquot (5 mL) of the resultant organic layer was pipetted out and transferred into an acid-washed 60 mL BOD bottle. Samples were then digested in a mixture (1:4 by volume) of concentrated trace-metal-grade nitric and sulfuric acids (5 mL) on a hot plate (~60 °C), which eliminated the organic solvents (methylene chloride and hexane) (Wagemann et al. 1997). Mercury content in the resultant aqueous layer was subsequently processed and analyzed as described above for fish samples.

Quality assurance – quality control

Mercury calibration standard solutions (0.1, 0.5, 1.0, 2.5, and 5.0 μg·L^{-1}), method and reagent blanks, and samples of the certified reference material (dogfish muscle tissue, DORM-2 with reported certified values of 4.64 ± 0.26 μg·g^{-1} for total Hg and 4.47 ± 0.32 μg·g^{-1} for methyl Hg; National Research Council Canada) were analyzed with each batch of samples. The calibration standard solutions were analyzed every 10 samples; when the deviation from the initial value was more than ± 10%, the instrument was recalibrated. The correlation coefficients of calibration curves were maintained at >0.999. Samples with the relative standard deviation between replicates of >15% were reanalyzed. The recovery rates of total Hg and organic (methyl) Hg from the DORM-2 samples were 95.6% ± 1.74% (n = 17) and 98.4% ± 2.15% (n = 23), respectively. All standard solutions and samples were prepared with NANOpure water. All glassware was soaked in 25% nitric acid overnight, rinsed once with deionized water, and then rinsed twice with NANOpure water.

Hg assimilation efficiencies

Methyl mercury (CH_3Hg) assimilation efficiency (AE) in the Hg mass-balance model is frequently assumed to be 0.8 for carnivorous fishes (Borgmann and Whittle 1992; Trudel et al. 2000). However, food consumption rates estimated by the Hg mass-balance model are sensitive to the error associated with AE (Trudel et al. 2000). Therefore, in the present study, prey-specific AE of dietary CH_3Hg in mummichogs previously estimated through a series of pulse-chase feeding experiments (Goto and Wallace 2009*a*) were incorporated into the Hg mass-balance model (Table 3), except for that of mollusks, which was derived from the literature (Pentreath 1976). In these experiments, *Palaemonetes* spp. were used for an estimation of the AE for decapods, *Nereis virens* for polychaetes, *Gammarus mucronatus* and *Leptochirus plumulosus* for amphipods, and *Chironomus dilutus* for aquatic insect larvae.

Dietary Hg uptake

Weighted averages of dietary Hg uptake ($AE\text{-}Hg_d$, ng·g^{-1}; Appendix A, Table A1) were estimated using population-specific pooled dietary data for each age class of mummichogs, prey-specific AEs, and site-specific organic Hg content in each prey as

$$(2)\quad AE \cdot Hg_d = \sum p_i \cdot AE_i \cdot Hg_{di}$$

where p_i is the proportion of the ith prey category in the diet by weight (Table 4), AE_i is the assimilation efficiency of CH_3Hg from the ith prey category (Table 3), and Hg_{di} is the organic Hg (CH_3Hg) concentration in the ith prey category (ng·g^{-1}).

Hg elimination rates

In estimating the daily elimination rate of Hg by fish in the field setting, the effect of the initial rapid loss of unassimilated Hg through egestion on Hg bioaccumulation is negligible (Trudel and Rasmussen 1997). Thus, only the slow component of Hg loss through excretion was incorporated into the mass-balance model (Trudel and Rasmussen 1997). Hg elimination (E) was estimated by an empirical model, which is independent of species or Hg whole-body burden (Trudel and Rasmussen 1997), as

$$(3)\quad E = \varphi \cdot W^{\beta} \cdot e^{\gamma T}$$

where W is fish body size (g), T is water temperature (°C), and φ, β, and γ are empirically derived constants (Table 3).

Hg loss through spawning

Annual average daily Hg loss through spawning (K) by adult mummichogs was estimated by the following model (Trudel et al. 2000):

$$(4)\quad K = Q \cdot GSI/\Delta t$$

where Q is the ratio of Hg in gonads (ng·g^{-1}) to Hg whole-

Table 3. Summary of parameter values used for the Hg mass-balance model.

Symbol	Parameter description	Value	Source
AE_d	Assimilation efficiency of CH_3Hg from decapods	0.55	Goto and Wallace (2009*a*)
AE_p	Assimilation efficiency of CH_3Hg from polychaetes	0.67	Goto and Wallace (2009*a*)
AE_a	Assimilation efficiency of CH_3Hg from amphipods	0.68	Goto and Wallace (2009*a*)
AE_d	Assimilation efficiency of CH_3Hg from insect larvae	0.81	Goto and Wallace (2009*a*)
AE_m	Assimilation efficiency of CH_3Hg from mollusks	0.25	Pentreath (1976)
φ	Coefficient of Hg elimination	0.0029	Trudel and Rasmussen (1997)
β	Allometric exponent of Hg elimination	–0.20	Trudel and Rasmussen (1997)
γ	Temperature coefficient of Hg elimination	0.066	Trudel and Rasmussen (1997)
GSI_m	Gonadosomatic index of male mummichogs (*Fundulus heteroclitus*)	0.037	Kneib and Stiven (1978); Leblanc et al. (1997); Shimizu (1997)
GSI_f	Gonadosomatic index of female mummichogs (*Fundulus heteroclitus*)	0.161	Kneib and Stiven (1978); Taylor (1986); Hsiao et al. (1994); Leblanc et al. (1997); Shimizu (1997); Hunter et al. (2007)

Table 4. Age-specific diet compositions of mummichogs (*Fundulus heteroclitus*) from Lemon Creek, Mill Creek, Richmond Creek, Main Creek, and Neck Creek.

			Prey taxa (% weight)*					
Study site	Age class	*n*	Decapoda	Isopoda	Amphipoda	Polychaeta	Insecta	Gastropoda
Lemon Creek	Age 2	21	64.31	3.36	1.05	3.02	1.11	0.59
	Age 3	23	43.66	1.25	0.00	1.78	0.76	29.11
Mill Creek	Age 2	27	53.62	0.00	3.51	1.51	0.60	2.19
	Age 3	17	58.52	0.68	2.88	0.38	1.83	4.34
Richmond Creek	Age 2	28	60.25	0.18	2.38	10.24	0.84	0.00
	Age 3	34	68.75	0.06	2.25	4.95	2.41	0.00
Main Creek	Age 2	42	45.00	1.24	8.57	12.99	1.95	0.00
	Age 3	30	6.82	8.11	0.00	21.56	0.00	19.08
Neck Creek	Age 2	37	55.63	0.13	7.17	11.90	0.00	0.00
	Age 3	31	53.77	3.33	8.23	21.43	0.46	0.00

*Source of the data: Goto (2009).

body burden (ng·g^{-1}), GSI is gonadosomatic index (g·g^{-1}) (Table 3), and Δt is 365 (days).

In general, the amount of Hg released through spawning by fish including mummichogs is substantially low, as gonadal Hg is a small fraction of Hg whole-body burden (Toppin et al. 1987; Baker Matta et al. 2001). Furthermore, the errors associated with the estimates of Q or GSI introduce little uncertainty (<5%) to the estimates of food consumption rates (Trudel et al. 2000). Therefore, in the current study, Q was estimated by using the concentration of Hg determined using pooled samples of gonads (n = ~50 to 100 per male or female) for each population (Hg_g, ng·g dry weight^{-1}), as described for the whole-body burdens (above).

Age determination and growth rate estimation

Age of mummichogs was determined using scales. Scale samples were first cleaned by soaking in a diluted detergent solution for a few minutes and rinsing with warm water. Then, each scale was mounted on a glass slide and aged by counting annuli under a stereomicroscope (40×) (DeVries and Frie 1996). All regenerated and distorted scale samples were discarded. Once age was determined, the image of each scale was captured using a digital microscope camera (Moticam 1000, Motic Group Co., Ltd.) mounted on a stereomicroscope, and the distance between the focus and each annulus at the anterio-lateral section was measured (±0.001 mm) (DeVries and Frie 1996) with digital imaging software (Motic Images Plus 2.0 ML, Motic Group Co., Ltd.). To increase precision, three to five scale samples per fish were aged and measured, and average radii were used for growth estimation. The relationship between age and total length was back-calculated using the Fraser–Lee method (DeVries and Frie 1996):

$$(5) \qquad L_i = (L_c - a)(S_i/S_c) + a$$

where L_i is back-calculated fish total length at the ith annulus (cm), L_c is fish total length at capture (cm), S_i is scale radius to the ith annulus (mm), S_c is total scale radius at capture (mm), and a is the Fraser–Lee correction factor (= the intercept of regression between the fish total length at capture and total scale radius at capture).

Weight-at-age of mummichogs was estimated by length (L) – weight (W) relationships determined for each population: $W = aL^b$, where a and b are constants. The parameters a and b were iteratively estimated using the Levenberg–Marquardt least squares algorithm. Then, specific growth rate (G, g·g^{-1}·day^{-1}) of an individual fish was determined using the following nonlinear equation (Ricker 1975):

$$(6) \qquad G = \ln(W_{t+\Delta t}/W_t) \cdot 1/\Delta t$$

where $W_{t+\Delta t}$ and W_t are weight (g wet weight) of mummichogs at time $t + \Delta t$ and t (days), respectively.

Estimation of growth conversion efficiency

Annual average growth conversion efficiency (GCE) (percentage of energy allocated to growth) of mummichogs was estimated by the following equation:

$$(7) \quad \%GCE = 100 \cdot G/C$$

where G and C are the growth and food consumption rate estimates of field-collected mummichogs (above), respectively.

Estimation of total metabolic costs

Total metabolic costs (M_T), which include standard metabolism, activity cost, and specific dynamic action (energy expended for food digestion and assimilation), were estimated using the following bioenergetics model (Hanson et al. 1997):

$$(8) \quad C = M_T + G + S + F + U$$

where C is food consumption rate, G is growth rate, S is gonadal growth, F is egestion, and U is excretion. All components in the bioenergetics model are expressed in g·g^{-1}·day^{-1} (Kitchell et al. 1977). This equation was solved for M_T by incorporating observed estimates of food consumption and growth and literature-derived estimates of gonadal loss (S = GSI·$W_f E_g$, where E_g is energy density of gonads; we assumed that the energy density of female gonads is 12.00% higher than that of somatic tissues, whereas the energy density of male gonads is the same as that of somatic tissues; Schultz and Conover 1997), egestion, and excretion (F = 12.75% and U = 11.72% of the consumed energy; Weisberg and Lotrich 1982*b*) into the bioenergetics model: $M_T = C - (G + S + F + U)$. All of the parameters in the bioenergetics model (g·g^{-1}·day^{-1}) were then converted to kJ·g^{-1}·day^{-1} using literature-derived energy densities: 4.60 kJ·g wet weight^{-1} for decapods, 1.70 kJ·g wet weight^{-1} for amphipods, 4.58 kJ·g wet weight^{-1} for polychaetes, 2.28 kJ·g wet weight^{-1} for gastropods (Steimle and Terranova 1985), and 2.74 kJ·g wet weight^{-1} for insects (Cummins and Wuycheck 1971), as well as 5.48 kJ·g wet weight^{-1} for mummichogs (Thayer et al. 1973).

Statistical analyses

Prior to analyses, data were arcsine- (for percentage data) or $\log_{10}$-transformed where necessary (Sokal and Rohlf 1995). Statistical significance of differences in annual average specific daily food consumption rates (g·g^{-1}·day^{-1}), specific growth rates (g·g^{-1}·day^{-1}), growth conversion efficiencies (%), and total metabolic rates (kJ·g^{-1}·day^{-1}) among populations of mummichogs were tested by three-way (site × age × sex) analysis of variance (ANOVA) at the significance level of $p < 0.05$. In addition to the site effect (i.e., habitat condition), because multivariate analysis of the AK benthic macroinfaunal community from our previous study showed that the community composition at one of the AK sites, Mill Creek, is similar to that of the reference site (Lemon Creek) and significantly different from other AK sites (Goto and Wallace 2010), we also tested the effect of benthic prey community (using three-way ANOVA) by grouping Lemon Creek and Mill Creek as non-prey-impoverished marsh (NPIM) sites, and Richmond Creek, Main Creek, and Neck Creek as prey-impoverished marsh (PIM) sites. If the results of ANOVAs were significant, Tukey's honestly significant difference (HSD) test was performed to test specific differences between the AK and reference sites, as well as between the NPIM and PIM sites. Due to uneven sample sizes among groups, a p value for each comparison was adjusted by Bonferroni correction (Sokal and Rohlf 1995).

Additionally, the relationship between growth rate and food consumption rate was also tested using linear regression. Due to heterogeneous slopes between the NPIM and PIM populations, we performed a nonparametric test by Pazzia et al. (2002) for significance of the difference between these populations. Briefly, we first fitted a simple linear regression model based on the PIM population data only and then performed a signed rank test to compare predicted values of growth rate of the NPIM population estimated from this regression model with observed values of the NPIM population. All statistical analyses were performed with STATISTICA (version 7.1, StatSoft®, Inc., Tulsa, Oklahoma).

Results

Growth rates and body sizes

Annual average daily growth rates of mummichogs varied from 0.0009 to 0.0037 g·g^{-1}·day^{-1} (Figs. 2*a* and 2*b*). Three-way ANOVA showed significant differences in specific growth rates among sites, as well as between age groups, but all interactions among site, age, and sex were also significant (Table 5). Tukey's HSD test among sites showed that the growth rates of age-2+ females from Lemon Creek (0.0037 g·g^{-1}·day^{-1}) were significantly higher than those from the other sites (~0.0013 g·g^{-1}·day^{-1} at Neck Creek and Main Creek to 0.0030 g·g^{-1}·day^{-1} at Mill Creek) (Fig. 2*a*). In contrast, age-2+ males from Lemon Creek had slightly lower growth rates than those from Neck Creek and Main Creek, whereas the growth rates of Mill Creek and Richmond Creek males were not significantly different from those of Lemon Creek males (Fig. 2*b*). The growth rate of age-3+ females from Lemon Creek (0.0009 g·g^{-1}·day^{-1}) was slightly lower than those of the other age-3+ females (~0.0014 g·g^{-1}·day^{-1}) (Fig. 2*a*), whereas there was no difference for age-3+ males among sites (~0.0009 g·g^{-1}·day^{-1}) (Fig. 2*b*).

When sites were pooled for benthic prey community, three-way ANOVA showed significant differences in growth rates of mummichogs only between age-2+ and age-3+ fish, whereas all interactions among benthic prey community, age, and sex (except for the one between benthic prey community and sex) were significant (Table 6). Age-2+ female and male fish showed contrasting trends; Tukey's HSD test showed that females of the PIM populations had significantly lower growth rates (0.0016 g·g^{-1}·day^{-1}) than those of the NPIM populations (0.0033 g·g^{-1}·day^{-1}) (Fig. 3*a*). On the other hand, males of the PIM populations had significantly higher growth rates (~0.0030 g·g^{-1}·day^{-1}) than those of the NPIM populations (~0.0023 g·g^{-1}·day^{-1}) (Fig. 3*b*). There was no significant difference in growth rates of age-3+ fish between NPIM and PIM.

Average weight-at-age of mummichogs varied from 3.07 to 5.51 g for age-2+ fish and from 4.99 to 10.3 g for age-

Fig. 2. Comparisons of bioenergetics of age-2+ (solid diamonds and circles) and age-3+ (shaded diamonds and circles) mummichogs (*Fundulus heteroclitus*) among sites (Lemon Creek, Mill Creek, Richmond Creek, Neck Creek, and Main Creek): daily specific growth rates ($g \cdot g^{-1} \cdot day^{-1}$) of (*a*) females and (*b*) males; daily specific food consumption rates ($g \cdot g^{-1} \cdot day^{-1}$) of (*c*) females and (*d*) males; growth conversion efficiency (%) of (*e*) females and (*f*) males; total metabolic rates ($kJ \cdot g^{-1} \cdot day^{-1}$) of (*g*) females and (*h*) males. Errors bars indicate ±1 standard error (SE). Different letters (uppercase for age 2+ and lowercase for age 3+) above or below each symbol indicate statistically significant differences among sites (Tukey's honestly significant difference (HSD) test with Bonferroni correction).

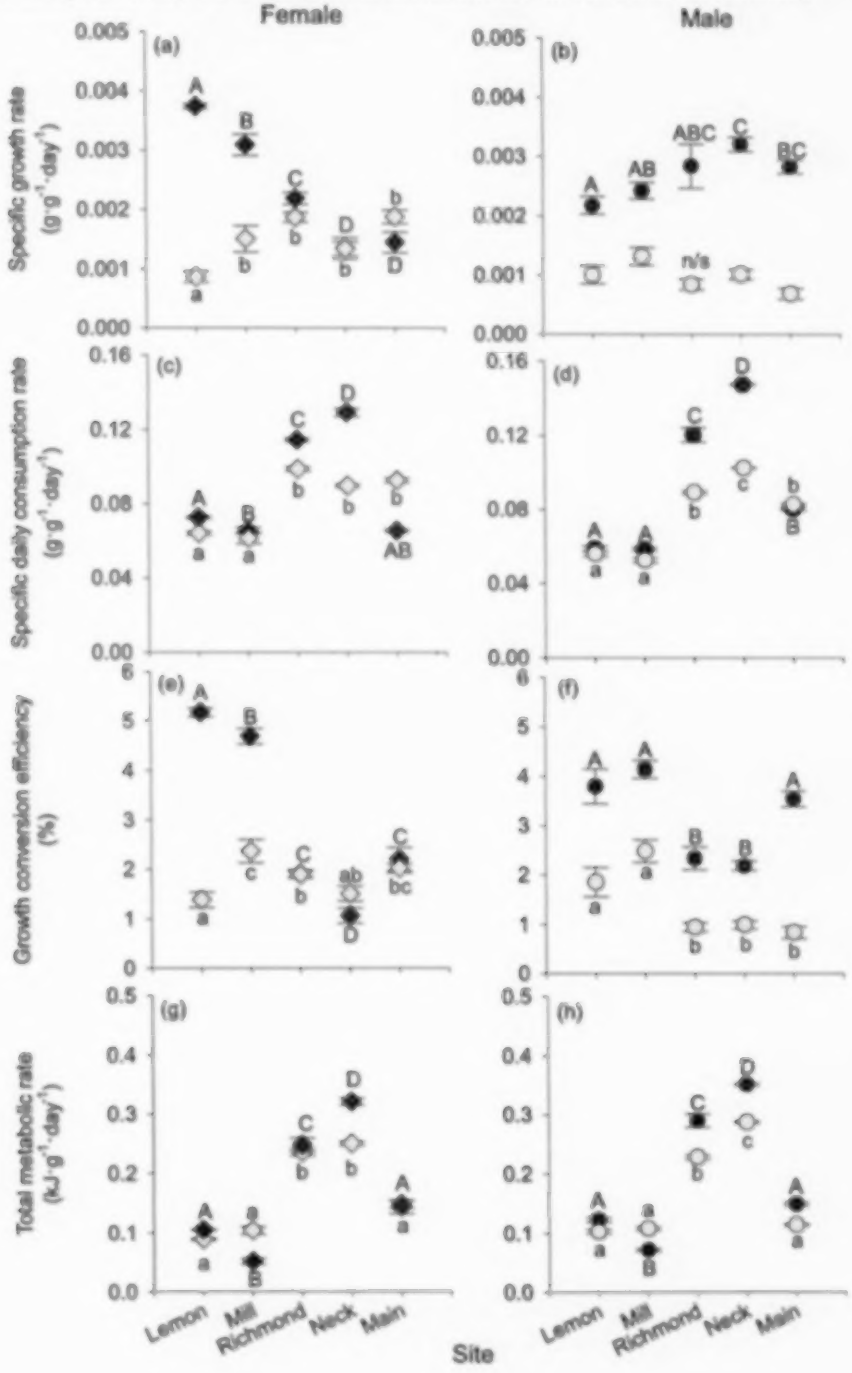


Table 5. Results of three-way analysis of variance (ANOVA) for the effects of site, age, and sex on mummichog (*Fundulus heteroclitus*) food consumption rate, growth rate, growth conversion efficiency, and total metabolic rate.

		SS	df	MS	F value	p value
Growth rate	Site	0.24	4	0.060	2.8	**0.030**
	Age	3.23	1	3.225	149.0	**<0.001**
	Sex	0.06	1	0.055	2.5	0.114
	Site × age	0.39	4	0.098	4.5	**0.002**
	Site × sex	0.35	4	0.088	4.1	**0.004**
	Age × sex	0.57	1	0.568	26.2	**<0.001**
	Site × age × sex	1.16	4	0.291	13.4	**<0.001**
	Residual	2.82	130	0.022		
Food consumption rate	Site	1.89	4	0.473	800.3	**<0.001**
	Age	0.08	1	0.076	127.9	**<0.001**
	Sex	0.01	1	0.005	8.6	**0.004**
	Site × age	0.20	4	0.050	84.3	**<0.001**
	Site × sex	0.09	4	0.021	36.1	**<0.001**
	Age × sex	0.01	1	0.010	17.1	**<0.001**
	Site × age × sex	0.03	4	0.006	10.9	**<0.001**
	Residual	0.08	130	0.001		
Growth conversion efficiency	Site	330.01	4	82.503	73.1	**<0.001**
	Age	285.25	1	285.254	252.8	**<0.001**
	Sex	1.24	1	1.244	1.1	0.296
	Site × age	18.86	4	4.714	4.2	**0.003**
	Site × sex	11.54	4	2.885	2.6	**0.042**
	Age × sex	11.99	1	11.990	10.6	**0.001**
	Site × age × sex	8.10	4	2.025	1.8	0.134
	Residual	146.71	130	1.129		
Total metabolic rate	Site	7.44	4	1.860	442.1	**<0.001**
	Age	0.01	1	0.008	1.8	0.182
	Sex	0.28	1	0.279	66.2	**<0.001**
	Site × age	0.60	4	0.150	35.6	**0.003**
	Site × sex	0.15	4	0.038	9.0	0.137
	Age × sex	0.10	1	0.098	23.2	0.090
	Site × age × sex	0.14	4	0.035	8.3	0.948
	Residual	0.55	130	0.004		

Note: SS, sum of squares; df, degrees of freedom; MS, mean square. Statistically significant differences are indicated in bold ($p < 0.05$).

3+ fish (Figs. 4*a* and 4*b*). There were significant differences in weight-at-age among sites (age 2+, $F_{[4,83]} = 7.18$, $p < 0.001$; age 3+, $F_{[4,107]} = 10.6$, $p < 0.001$; Figs. 4*a* and 4*b*), whereas females and males both showed similar trends for age-2+ and age-3+ fish (age 2+, $F_{[4,83]} = 1.01$, $p = 0.31$; age 3+, $F_{[4,107]} = 1.12$, $p = 0.23$; Figs. 4*a* and 4*b*). The interaction between site and sex was not significant for age-2+ or age-3+ fish (age 2+, $F_{[4,83]} = 1.48$, $p = 0.15$; age 3+, $F_{[4,107]} = 1.75$, $p = 0.21$). Age-2+ fish from Richmond Creek, Mill Creek, and Neck Creek were slightly heavier than those from the other sites (Figs. 4*a* and 4*b*). Similarly, age-3+ fish from Richmond Creek, Mill Creek, and Neck Creek were substantially heavier than those from Lemon Creek (Figs. 4*a* and 4*b*).

Food consumption rates

Annual average daily food consumption rates of age-2+ and age-3+ male and female mummichogs varied from 0.052 to 0.147 $g \cdot g^{-1} \cdot day^{-1}$ (Figs. 2*c* and 2*d*). Three-way ANOVA showed significant differences in food consumption rates of mummichogs among all groups (site, age, and sex), but all interactions among site, age, and sex were also significant (Table 5). Tukey's HSD test among sites showed that specific food consumption rates of age-2+ female fish from Lemon Creek (0.072 $g \cdot g^{-1} \cdot day^{-1}$) were slightly higher than those from Mill Creek (0.065 $g \cdot g^{-1} \cdot day^{-1}$) but significantly lower than those from Richmond Creek (0.114 $g \cdot g^{-1} \cdot day^{-1}$) and Neck Creek (0.129 $g \cdot g^{-1} \cdot day^{-1}$) (Fig. 2*c*). The food consumption rates of Main Creek female fish (0.067 $g \cdot g^{-1} \cdot day^{-1}$) were not significantly different from those of either Lemon Creek or Mill Creek fish (Fig. 2*c*). A similar trend was also observed for age-2+ males: the highest food consumption rates were observed at Neck Creek (0.147 $g \cdot g^{-1} \cdot day^{-1}$), which was followed by Richmond Creek (0.120 $g \cdot g^{-1} \cdot day^{-1}$) > Main Creek (0.080 $g \cdot g^{-1} \cdot day^{-1}$) > Lemon Creek = Mill Creek (~0.058 $g \cdot g^{-1} \cdot day^{-1}$) (Fig. 2*d*). The food consumption rates of both age-3+ females and males from Lemon Creek and Mill Creek were similar to those of age-2+ fish from the corresponding sites (~0.06–0.07 $g \cdot g^{-1} \cdot day^{-1}$) and significantly lower than age-3+ fish from Richmond Creek, Neck Creek, and Main Creek (~0.09–0.10 $g \cdot g^{-1} \cdot day^{-1}$) (Figs. 2*c* and 2*d*).

When sites were pooled for benthic prey community, three-way ANOVA showed significant differences in food

Table 6. Results of three-way analysis of variance (ANOVA) for the effects of benthic prey community, age, and sex on mummichog (*Fundulus heteroclitus*) food consumption rate, growth rate, growth conversion efficiency, and total metabolic rate.

		SS	df	MS	*F* value	*p* value
Growth rate	Benthos	0.01	1	0.01	0.4	0.539
	Age	4.01	1	4.01	154.0	**<0.001**
	Sex	0.02	1	0.02	0.8	0.374
	Benthos × age	0.27	1	0.27	10.3	**0.002**
	Benthos × sex	0.06	1	0.06	2.4	0.126
	Age × sex	0.34	1	0.34	13.1	**<0.001**
	Benthos × age × sex	1.21	1	1.21	46.6	**<0.001**
	Residual	3.70	142	0.03		
Food consumption rate	Benthos	1.67	1	1.67	272.0	**<0.001**
	Age	0.09	1	0.08	14.1	**<0.001**
	Sex	0.01	1	0.01	1.3	0.250
	Benthos × age	0.01	1	0.01	2.4	0.125
	Benthos × sex	0.07	1	0.07	11.0	**0.001**
	Age × sex	0.01	1	0.01	1.4	0.246
	Benthos × age × sex	0.01	1	0.01	1.2	0.279
	Residual	0.87	142	0.01		
Growth conversion efficiency	Benthos	42.10	1	42.10	3.1	**0.032**
	Age	238.50	1	238.50	68.3	**<0.001**
	Sex	5.95	1	5.95	1.5	0.221
	Benthos × age	7.24	1	7.24	1.8	0.177
	Benthos × sex	3.93	1	3.93	1.0	0.319
	Age × sex	0.24	1	0.24	0.1	0.806
	Benthos × age × sex	12.38	1	12.38	3.1	0.078
	Residual	558.64	142	3.93		
Total metabolic rate	Benthos	4.77	1	4.77	135.0	**<0.001**
	Age	0.06	1	0.06	1.6	0.209
	Sex	0.48	1	0.48	13.6	**<0.001**
	Benthos × age	0.17	1	0.17	4.8	0.644
	Benthos × sex	0.01	1	0.01	0.2	0.657
	Age × sex	0.16	1	0.16	4.6	0.103
	Benthos × age × sex	0.01	1	0.01	0.2	0.685
	Residual	5.01	142	0.04		

Note: SS, sum of squares; df, degrees of freedom; MS, mean square. Statistically significant differences are indicated in bold ($p < 0.05$).

consumption rates of mummichogs between NPIM and PIM sites and between age-2+ and age-3+ fish, whereas only the interaction between benthic prey community and sex was significant (Table 6). Both age-2+ and age-3+ fish showed similar trends; Tukey's HSD test showed that the PIM populations had significantly higher food consumption rates (~0.10 $g \cdot g^{-1} \cdot day^{-1}$) than the NPIM populations (~0.06 $g \cdot g^{-1} \cdot day^{-1}$) (Figs. 3*c* and 3*d*).

Growth conversion efficiency

Annual average growth conversion efficiencies of mummichogs varied from 0.95% to 5.2% (Figs. 2*e* and 2*f*). Three-way ANOVA showed significant differences in conversion efficiencies among sites, as well as between age groups, but the interaction between these two factors was also significant (Table 5). Tukey's HSD test among sites showed that the conversion efficiencies of age-2+ females from Lemon Creek and Mill Creek (~4.8%) were more than twofold higher than those of Richmond Creek, Main Creek (~2.0%), and Neck Creek (1.1%) (Fig. 2*e*). The conversion efficiencies of age-2+ males from Lemon Creek, Mill Creek, and Main Creek (~3.8%) were approximately twofold higher than those of Richmond Creek and Neck Creek (~2.3%) (Fig. 2*f*). Age-3+ females from Lemon Creek (1.4%) had a slightly lower conversion efficiency than those from Richmond Creek (1.9%), Main Creek (2.0%), and Mill Creek (2.4%) (Fig. 2*e*), whereas the conversion efficiencies of age-3+ males from Lemon Creek and Mill Creek (~2.2%) were approximately twofold higher than those of Richmond Creek, Neck Creek, and Main Creek (~0.93%) (Fig. 2*f*).

When sites were pooled for benthic prey community, three-way ANOVA showed significant differences in growth conversion efficiencies of mummichogs only between the NPIM and PIM sites and age-2+ and age-3+ fish (Table 6), whereas there was no interaction effect between these two factors (Table 6). Tukey's HSD test showed that with the exception of age-3+ females, the PIM populations had significantly lower conversion efficiencies (0.93%–2.7%) than the NPIM populations (2.1%–4.8%) (Fig. 3*e*). Additionally, the relationships between growth rates (G) and food consumption rates (C) were significant for both the NPIM ($\log_{10}G = 0.67\log_{10}C - 2.0$, $R^2 = 0.34$, $F_{[67]} = 4.37$, $p = 0.041$) and PIM ($\log_{10}G = 0.73\log_{10}C - 2.3$, $R^2 = 0.46$, $F_{[83]} = 11.67$, $p < 0.001$) populations (Fig. 5). A signed rank test showed that growth rates of the NPIM population were significantly higher than those of the PIM population over varying consumption rates ($T_{[67]} = 680.0$, $p = 0.001$) (Fig. 5).

Fig. 3. Comparisons of bioenergetics of age-2+ (solid diamonds and circles) and age-3+ (shaded diamonds and circles) mummichogs (*Fundulus heteroclitus*) between non-prey-impoverished marsh sites (Lemon Creek and Mill Creek) and prey-impoverished marsh sites (Richmond Creek, Neck Creek, and Main Creek): daily specific growth rates ($g \cdot g^{-1} \cdot day^{-1}$) of (*a*) females and (*b*) males; daily specific food consumption rates ($g \cdot g^{-1} \cdot day^{-1}$) of (*c*) females and (*d*) males; growth conversion efficiency (%) of (*e*) females and (*f*) males; total metabolic rates ($kJ \cdot g^{-1} \cdot day^{-1}$) of (*g*) females and (*h*) males. Errors bars indicate ±1 standard error (SE). Different letters (uppercase for age 2+ and lowercase for age 3+) above or below each symbol indicate statistically significant differences between sites (Tukey's honestly significant difference (HSD) test with Bonferroni correction).

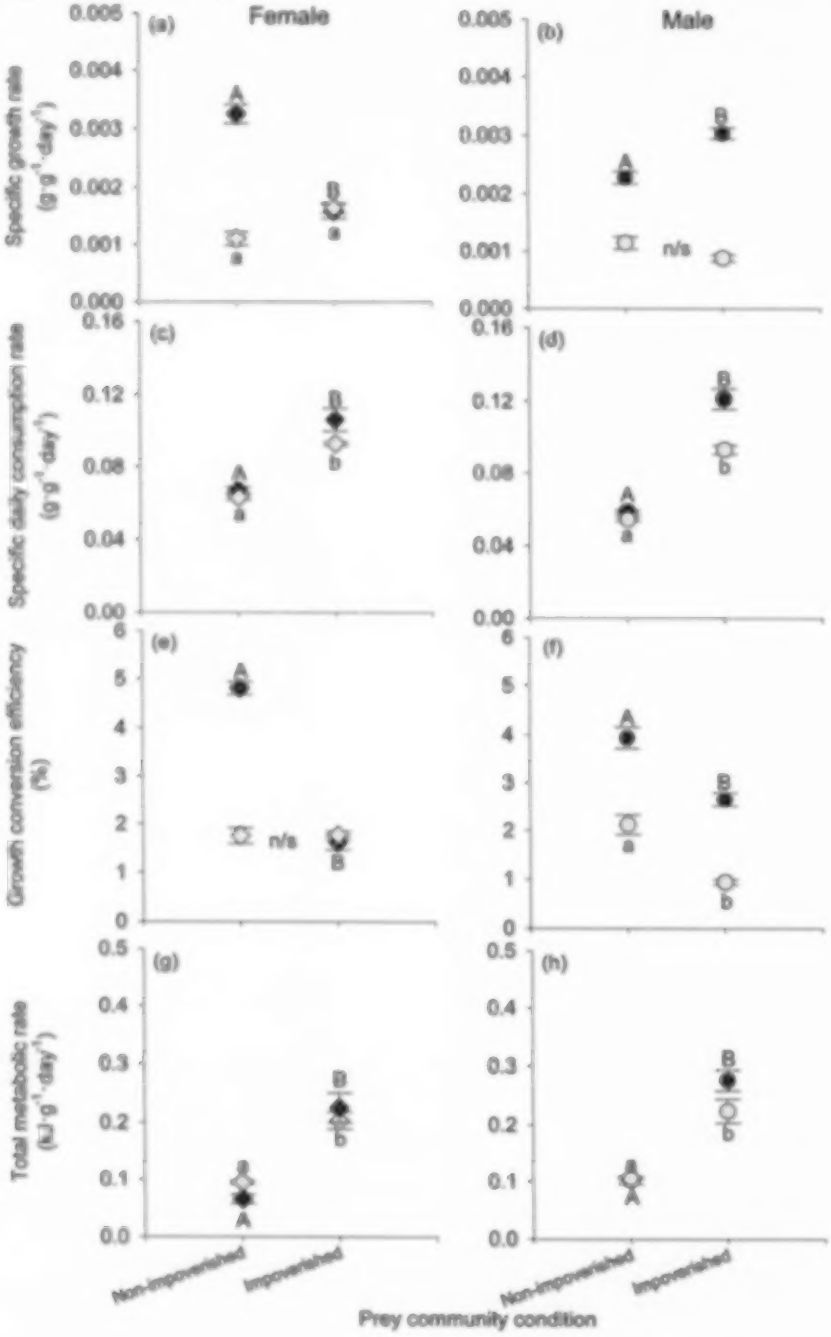
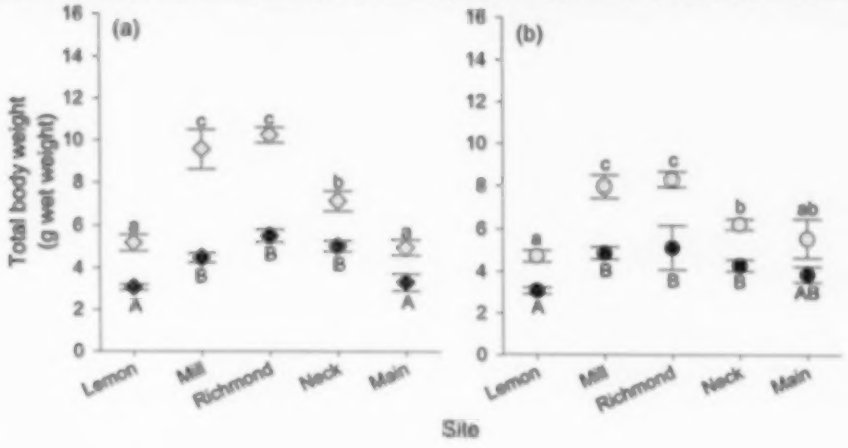


Fig. 4. Comparisons of body size (g wet weight) of age-2+ (solid diamonds and circles) and age-3+ (shaded diamonds and circles) mummichogs (*Fundulus heteroclitus*) from Lemon Creek, Mill Creek, Richmond Creek, Neck Creek, and Main Creek: (*a*) females and (*b*) males. Errors bars indicate ±1 standard error (SE). Different letters (uppercase for age 2+ and lowercase for age 3+) above or below each symbol indicate statistically significant differences among sites (Tukey's honestly significant difference (HSD) test with Bonferroni correction).



Total metabolic costs

Indirect estimates of annual average total metabolic costs of mummichogs varied from 0.05 to 0.35 kJ·g^{-1}·day^{-1} (Figs. 2*g* and 2*h*). Three-way ANOVA showed significant differences in specific metabolic costs only among sites (Table 5); the interaction between site and sex was also significant (Table 5). Additionally, with the exception of males and females from Lemon Creek, there was a significant relationship between total metabolic cost (kJ·day^{-1}) and body size (g wet weight); the metabolic costs significantly increased with increasing body size (Figs. 6*a*–6*e*). Tukey's HSD test among sites showed that the total metabolic rate of age-2+ females from Neck Creek (0.321 kJ·g^{-1}·day^{-1}) was more than fourfold higher than those of the other females (~0.074 kJ·g^{-1}·day^{-1}), except for those from Richmond Creek (0.248 kJ·g^{-1}·day^{-1}) (Fig. 2*g*). The total metabolic rates of age-2+ males from Mill Creek (0.072 kJ·g^{-1}·day^{-1}), as well as from Lemon Creek and Main Creek (~0.137 kJ·g^{-1}·day^{-1}), were significantly lower than those of Richmond Creek and Neck Creek (~0.321 kJ·g^{-1}·day^{-1}) (Fig. 2*h*). The metabolic rates of both age-3+ females and males from Richmond Creek and Neck Creek (~0.25 kJ·g^{-1}·day^{-1}) were significantly higher than those of Lemon Creek, Mill Creek, and Main Creek (~0.11 kJ·g^{-1}·day^{-1}) (Figs. 2*g* and 2*h*).

When sites were pooled for benthic prey community, three-way ANOVA showed significant differences in specific total metabolic costs of mummichogs only between the NPIM and PIM sites, whereas there was a significant interaction effect between benthic prey community and sex (Table 6). Both male and female fish showed similar trends; Tukey's HSD test showed that PIM populations had significantly higher total metabolic costs (~0.26 kJ·g^{-1}·day^{-1}) than NPIM populations (~0.11 kJ·g^{-1}·day^{-1}) (Figs. 3*g* and 3*h*).

Discussion

The findings from the present study suggest that energetic responses of mummichogs were more related to altered benthic prey community than to habitat degradation of AK salt marshes. PIM (Richmond Creek, Main Creek, and Neck Creek) populations of mummichogs clearly displayed elevated metabolic rates, but they also had unexpectedly higher food consumption rates relative to NPIM (Mill Creek and Lemon

Fig. 5. Relationships between food consumption rates (g·g^{-1}·day^{-1}) and growth rates (g·g^{-1}·day^{-1}) of age-2+ and age-3+ female and male mummichogs (*Fundulus heteroclitus*). Errors bars indicate ±1 standard error (SE). A broken line indicates least squares regression for Lemon Creek and Mill Creek (non-prey-impoverished marsh sites, shaded circles). A solid line indicates least squares regression for Richmond Creek, Main Creek, and Neck Creek (prey-impoverished marsh sites, solid circles). Dotted lines indicate 95% confidence limit for least squares regression.

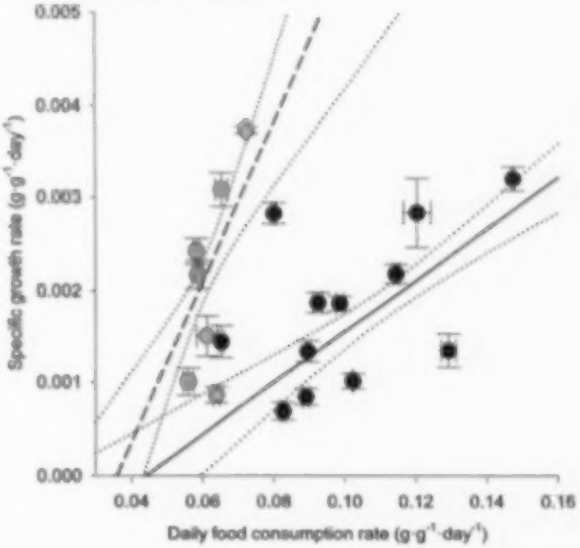
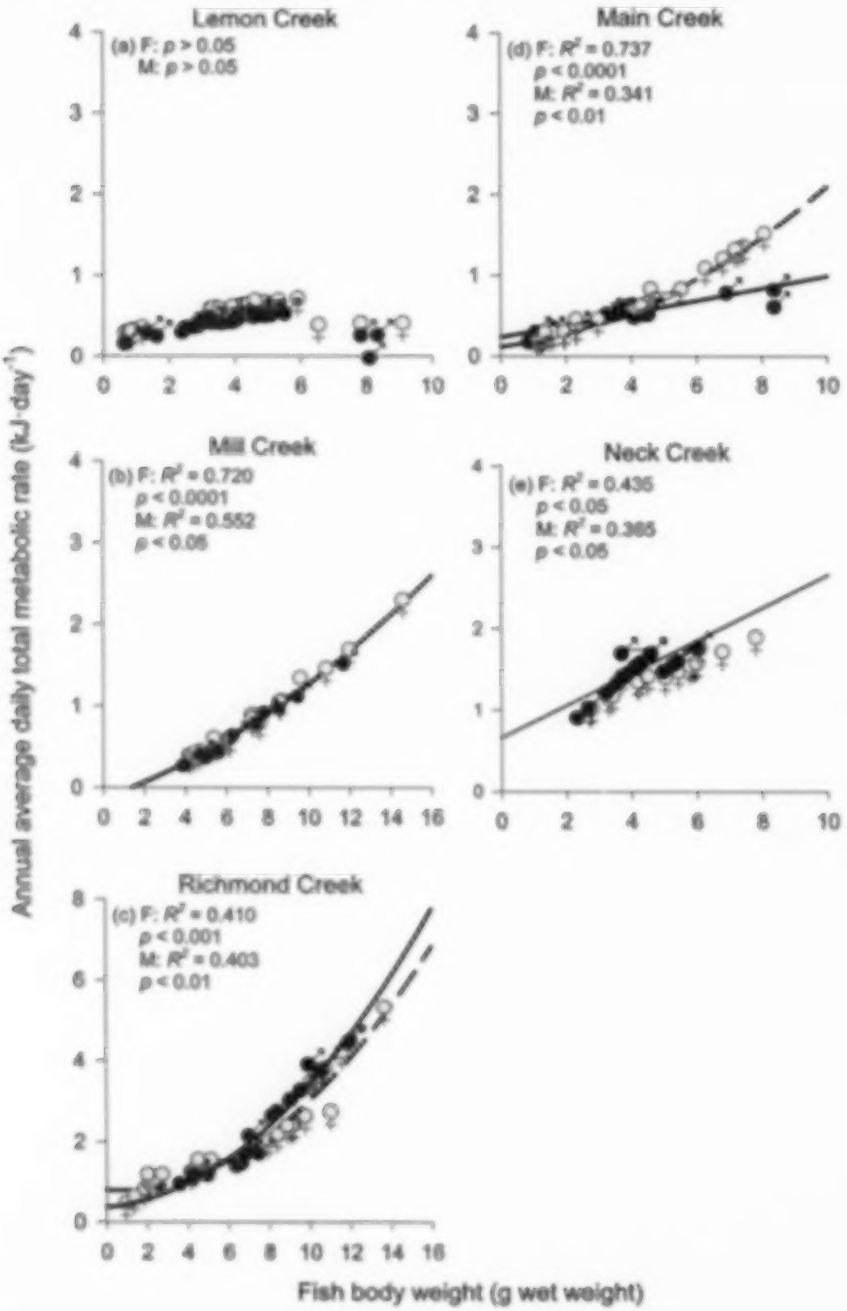


Fig. 6. Population-specific relationships between total metabolic rates (kJ·day^{-1}) and body size (g wet weight) of female and male mummichogs (*Fundulus heteroclitus*) from (*a*) Lemon Creek, (*b*) Mill Creek, (*c*) Richmond Creek, (*d*) Main Creek, and (*e*) Neck Creek. Shaded ♀ indicates females and solid ♂ indicates males. Broken lines indicate least squares regression for females. Solid lines indicate least squares regression for males.



Creek) populations. Despite their enhanced food consumption, however, the PIM populations still exhibited reduced growth conversion efficiency relative to the NPIM populations.

Energy allocation to growth in pollution-tolerant fish

Assimilated energy from food is normally allocated to growth (somatic and (or) gonadal) after accounting for es-

sential metabolic activities (Hanson et al. 1997). Because of its sensitivity to external (e.g., physicochemical properties of the environment), as well as internal (e.g., physiological processes), conditions, individual growth of fish is an ecologically relevant, integrative indicator frequently used in the assessment of fish habitat quality (Weatherley 1990; Beyers and Rice 2002). Thus, examining how habitat degradation and altered trophic structure influence potential energy availability to growth may reveal the mechanisms underlying alterations in fish life history traits in polluted habitats (Beyers et al. 1999*a*, 1999*b*). Increased metabolic costs can, for instance, be compensated for by re-allocation of energy that is potentially available to growth. In the current study, however, only age-2+ female mummichogs from the AK sites had substantially (up to 60%) reduced growth rates. In addition to altered energy availability to somatic growth, there may also have been alteration in energy allocation to gonadal growth of the AK populations of female mummichogs. For instance, Toppin et al. (1987) have suggested that negative somatic growth observed in female adult mummichogs living in chemically polluted habitats might be associated with their energetic investment in embryonic resistance to chemical pollutants. Further studies are, however, still required to elucidate mechanisms of how living in polluted habitats alters the processes of energy allocation to reproduction and recruitment of tolerant fishes.

Energy allocation to growth may be indirectly maintained by flexible foraging habits (Calow 1991; Health 1995). Growth of some fishes appears to be influenced little by alterations in prey resources in polluted habitats (e.g., reduced abundance or disappearance of sensitive large-bodied species; Steimle 1994; Clements and Rees 1997). The flexible feeding habit thus may be considered as an adaptive strategy (trophic plasticity) to acquire sufficient energy to maintain growth, adjusting to fluctuations in prey availability (Gerking 1994; Woodward et al. 2005). In the current study, despite their elevated metabolism, individual growth rates of age-2+ males and age-3+ females as well as males (unlike those of age-2+ females) of the AK populations of mummichogs were similar to or slightly higher than those of the reference (Lemon Creek) population. Furthermore, average weight-at-age of some of the AK populations was also larger than that of the reference population. Growth enhancement has previously been observed in fishes living in degraded habitats, most likely as a result of indirect ecological responses to altered trophic structures (e.g., reduced competition, as well as increased availability of tolerant prey species; Barlaup et al. 1989; Ryan and Harvey 1980; Henshel et al. 2006). Moreover, an ontogenetic shift in growth responses by female mummichogs in the current study may have been associated with size-dependent feeding ecology (Campbell et al. 2003). Even with progressively increasing metabolic costs with increasing body size (as observed in the current study), the efficiency to capture relatively larger prey organisms such as decapods is likely to improve as mummichogs grow, which may reduce the energetic cost of foraging activity to a certain extent (Sherwood et al. 2002).

Metabolic costs of fish living in polluted environments

Proportions (%) of energy allocated to total metabolism (= standard metabolism + active metabolism + specific dynamic action) of both age-2+ and age-3+ mummichogs were considerably varied among populations (48.8%–72.7%). Mummichogs from two of the northern AK sites (Richmond Creek and Neck Creek) consistently had two- to three-fold higher total metabolic rates ($kJ \cdot g^{-1} \cdot day^{-1}$) than those from the reference site (Lemon Creek). Energetic expenditures of fish living in chronically degraded habitats tend to be elevated, partly due to the increased metabolic activities induced by physiological impairments (Rajotte and Couture 2002; Couture and Kumar 2003). Many aquatic organisms including fish use a variety of physiological and biochemical processes to ameliorate adverse effects of anthropogenic stressors, which are likely to increase metabolic costs (Calow 1991; Moiseenko 2002).

However, mummichogs from the southern AK site, Mill Creek (where sediment-associated metal concentrations were substantially elevated), had total metabolic rates similar to those from the reference site. Incidentally, benthic macroinfaunal (a primary prey resource of mummichogs) community composition at Mill Creek is also similar to that of the reference site (Goto and Wallace 2010). Thus, when analysis was performed on the PIM (Richmond Creek, Main Creek, and Neck Creek) populations vs. the NPIM (Mill Creek and Lemon Creek) populations, the results showed that the PIM populations had consistently higher metabolic costs than the NPIM populations. These results suggest that indirect pollution effects (i.e., altered prey availability) may be a more important factor for bioenergetics of tolerant fish such as mummichogs than direct effects (i.e., pollutant toxicity) in chronically polluted environments. In the current study, however, only the data on select sediment-associated metal concentrations were available as pollution gradients, but AK has been polluted with a variety of chemical pollutants (e.g., PCBs; Adams and Benyi 2003). For instance, Vitaliano and Bejda (2001) have shown a clear difference in the health of ribbed mussels (*Geukensia demissa*) between the northern AK salt marshes and Mill Creek. Ribbed mussels from Mill Creek had considerably higher growth rate, larger body size, and better body condition (e.g., higher body weight – shell weight ratio) than those from the northern AK sites (Vitaliano and Bejda 2001). It is thus also possible that the northern AK salt marshes may be more degraded than the southern region.

Elevated metabolism is also frequently associated with alterations in fish behavioral activities (e.g., Ryan and Harvey 1980; Barlaup et al. 1989; Tucker and Rasmussen 1999). Anthropogenic stressors such as chemical pollutants can directly affect fish foraging behaviors (i.e., energy intake, e.g., Smith and Weis (1997) and Weis et al. (2001)) and indirectly influence fish energetics (Handy et al. 1999; Campbell et al. 2002). For instance, Weis et al. (2001) have demonstrated that the capacity of mummichogs to capture decapods (*Palaemonetes pugio*) was considerably compromised in chemically polluted salt marshes of the Northwest Atlantic coast. The northern AK or PIM populations of mummichogs in the current study may thus also have been forced to use an energetically inefficient feeding strategy, i.e., foraging for prey that are small but relatively easy to capture (= a high predator–prey body size ratio; Boisclair and Rasmussen 1996; Pazzia et al. 2002). In fact, one of the PIM (Main Creek) populations appeared unable to shift to a

decapod-dominated diet and continued to rely on smaller prey such as polychaetes (which comprised more than 90% of benthic macroinfaunal community) until their total length reached 80+ mm (Goto 2009).

Energetic strategy of pollution-tolerant fish

In highly urbanized habitats, some fishes can compensate for elevated energy expenditures using adaptive strategies including a reduction in behavioral activities (Handy et al. 1999), a re-allocation of stored energy (e.g., lipids and carbohydrates; Campbell et al. 2002; Levesque et al. 2002), and an increase in food consumption (Beyers and Rice 2002). Laboratory studies have suggested that reduced activities of stressed fish are a compensatory mechanism for elevated standard metabolism (Handy et al. 1999; Campbell et al. 2002). For instance, Handy et al. (1999) have indicated that a decrease in behavioral activities (e.g., swimming) by rainbow trout (*Oncorhynchus mykiss*) exposed to dietary copper appeared to be related to increased metabolic costs and decreased liver lipid reserves, suggesting that energy re-allocation was partly associated with altered behavioral activities. Smith and Weis (1997) have also experimentally demonstrated that mummichogs from polluted salt marshes had reduced attempts to capture decapod prey, which may be considered an adaptive strategy to reduce energy expenditure.

In contrast to the findings from the laboratory studies, daily food consumption rate estimates in the current study revealed that the northern AK or PIM populations of mummichogs with elevated total metabolism consumed two- to three-fold more food than the NPIM populations. Annual average daily food consumption rates of mummichogs estimated with the mercury mass balance model technique (~0.06–0.15 $g \cdot g^{-1} \cdot day^{-1}$) in the current study were also consistent with the field estimates using the gut evacuation method (~0.13 $g \cdot g^{-1} \cdot day^{-1}$) by Weisberg et al. (1981), as well as the laboratory estimates (~0.043 $g \cdot g^{-1} \cdot day^{-1}$) by Weisberg and Lotrich (1982*a*). Energy expended for foraging activities is often directly related to consumed energy, as well as stored energy (Handy et al. 1999; Campbell et al. 2002). The substantial difference in food consumption rates suggests that the PIM populations of mummichogs may have increased their energy intake to compensate for elevated metabolic costs, instead of conserving energy by reducing activities. The compensatory food consumption by mummichogs has also been observed in a study by Weisberg and Lotrich (1982*b*) in which fish fed with low-energy-content prey (*Uca* spp.) consumed more food than those fed with high-energy-content prey (*Palaemonetes* spp. and *Nereis* spp.). The energy-based adaptive strategy of food consumption may play an important role for wild populations of fishes in chronically degraded environments, especially when prey availability is also concurrently altered. In AK, reduced nekton species diversity (i.e., lower competition and predation risk) and increased abundances of tolerant benthic macroinvertebrate species (i.e., higher prey availability; Goto and Wallace 2010) may have contributed to the use of compensatory food consumption by mummichogs.

However, despite increased food consumption (i.e., foraging activity), the PIM (the northern AK) populations of mummichogs in the present study generally did not show a proportional increase in growth rate, which resulted in substantially lower (as much as 80%) growth conversion efficiencies than those of the NPIM populations. This decrease in energy conversion efficiency also suggests that growth enhancement observed in some of the PIM populations may have been a by-product of compensatory food consumption presumably induced by elevated metabolism (i.e., overcompensation). Because foraging activities can be energetically costly, the cost associated with increasing foraging activities (e.g., elevated metabolism) may eventually surpass the benefits (i.e., energy gain). Actively foraging fishes such as mummichogs thus may require balancing energy gained from food consumption and energy expended for foraging activities to maximize energy conversion efficiency and perhaps maintain growth rate (Handy et al. 1999; Campbell et al. 2002).

Implications of altered bioenergetics of mummichogs in polluted salt marshes

The AK populations of mummichogs appeared to be capable of adjusting food consumption rates to compensate for elevated metabolic costs and to maintain growth to a certain degree. However, they were unable to efficiently convert consumed energy to growth in these highly polluted habitats. A decrease in energy conversion efficiency at the individual level can lead to population-level impacts, including shifts in recruitment, productivity, and age–size structure (Carlisle 2000; Sherwood et al. 2000). Moreover, stress-induced reduction in energy conversion efficiency at one trophic level can also cascade through trophic chains (e.g., reduced trophic efficiency). Inefficient energy fluxes in stressed ecosystems have been documented frequently (Clements and Newman 2002) and could ultimately result in "energetic bottlenecks" at the community level (Nisbet et al. 1997; Carlisle 2000; Sherwood et al. 2000) and the potential disappearance of large-bodied apex predators (Clements and Newman 2002).

The importance of mummichogs as a conduit of energy and production transport to open coastal environments has been well recognized (Teo and Able 2003). Tidal salt marshes are a highly productive environment (Levin et al. 2001), supporting adjacent coastal ecosystems (Deegan et al. 2000). It has been suggested that the exportation of salt marsh production is mediated through trophic pathways (Kneib 1997); the spatial movement of marsh production toward open coastal habitats is intimately associated with energy flow through trophic interactions between resident and migrant species, as well as tidal migrations of nekton ("trophic relay"; Kneib 1997). Reduced energy conversion efficiencies in highly abundant resident fishes such as mummichogs observed in the current study are, therefore, likely to have further implications for overall trophic efficiencies in coastal ecosystems (e.g., potential disruption in trophically mediated processes).

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Appendix A

Table A1. Average body size at time t (W_t) and $t + \Delta t$ ($W_{t+\Delta t}$), mercury concentration in fish at time t (Hg_t) and $t + \Delta t$ ($Hg_{t+\Delta t}$), and dietary mercury uptake (assimilated mercury concentration weighted by proportions of prey category in the diet) by fish ($\Sigma p \cdot \alpha \cdot Hg_t$).

Site	Age	Sex	W_t (g)	$W_{t+\Delta t}$ (g)	Hg_t (ng·g^{-1})	$Hg_{t+\Delta t}$ (ng·g^{-1})	$\Sigma p \cdot \alpha \cdot Hg_t$ (ng·g^{-1})
Lemon Creek	2	M	1.45 (0.421, 5)	3.25 (0.199, 8)	71.18 (3.44, 5)	97.15 (2.91, 8)	25.10
		F	0.85 (0.089, 9)	3.33 (0.081, 5)	96.85 (4.76, 9)	101.09 (4.92, 5)	25.10
	3	M	3.25 (0.199, 8)	4.76 (0.278, 6)	97.15 (2.91, 8)	96.25 (2.21, 6)	18.81
		F	3.33 (0.081, 5)	4.61 (0.157, 9)	101.09 (4.92, 5)	110.12 (10.28, 9)	18.81
Mill Creek	2	M	2.00 (0.292, 6)	4.90 (0.286, 8)	56.65 (1.25, 6)	69.04 (1.22, 8)	19.12
		F	1.70 (0.211, 7)	5.36 (0.423, 5)	56.10 (1.10, 7)	75.13 (2.20, 5)	19.12
	3	M	4.90 (0.286, 8)	8.00 (0.538, 7)	69.04 (1.22, 8)	82.33 (2.30, 7)	21.58
		F	5.36 (0.423, 5)	9.58 (0.939, 8)	75.13 (2.20, 5)	97.07 (4.88, 8)	21.58
Richmond Creek	2	M	1.75 (0.351, 9)	5.14 (1.04, 5)	115.54 (2.18, 9)	136.60 (6.45, 5)	20.17
		F	2.01 (0.250, 8)	4.47 (0.217, 8)	117.11 (1.55, 8)	132.43 (1.35, 8)	20.17
	3	M	5.14 (1.04, 5)	7.02 (0.325, 6)	136.60 (6.45, 5)	148.26 (2.02, 6)	24.56
		F	4.47 (0.217, 8)	8.87 (0.317, 7)	132.43 (1.35, 8)	159.75 (1.97, 7)	24.56
Main Creek	2	M	1.19 (0.091, 4)	3.37 (0.140, 11)	243.26 (1.18, 4)	214.93 (1.82, 11)	41.61
		F	1.34 (0.091, 6)	2.30 (0.187, 8)	166.79 (1.17, 6)	179.07 (2.39, 8)	41.61
	3	M	3.37 (0.140, 11)	4.42 (0.223, 8)	214.93 (1.82, 11)	201.32 (2.90, 8)	31.13
		F	2.30 (0.187, 8)	4.59 (0.242, 7)	179.07 (2.39, 8)	208.33 (3.09, 7)	31.13
Neck Creek	2	M	1.16 (0.180, 8)	3.80 (0.170, 6)	296.23 (1.28, 8)	284.34 (0.457, 6)	40.91
		F	2.21 (0.194, 4)	3.69 (0.263, 8)	299.49 (3.38, 4)	271.48 (4.07, 8)	40.91
	3	M	3.80 (0.170, 6)	5.52 (0.217, 10)	284.34 (0.457, 6)	280.70 (0.361, 10)	48.43
		F	3.69 (0.263, 8)	6.07 (0.302, 9)	271.48 (4.07, 8)	245.04 (2.32, 9)	48.43

Note: Values in parentheses indicate standard error and sample size, respectively.

Large effective population size and temporal genetic stability in Atlantic cod (*Gadus morhua*) in the southern Gulf of St. Lawrence

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Abstract: Worldwide, many commercial fish stocks have experienced dramatic declines due to overfishing. Such fisheries-induced population reductions could potentially erode the genetic diversity of marine fish populations. Based on analyses of DNA extracted from archived and contemporary samples, this paper compares the genetic variability at nine microsatellite loci in a Canadian population of Atlantic cod (*Gadus morhua*) over 80 years, spanning from before the fishery intensified to now when the population is at historically low abundance. Extensively validated genetic data from the temporally spaced samples were used to estimate the effective population size. Over the period, we observed no loss of either heterozygosity or allelic diversity. Several of the estimation methods applied could not distinguish the effective population size from infinity, and the lower 95% confidence limit on estimates was generally >500, suggesting that the effective population size is probably considerably larger than this. Hence, it appears that the southern Gulf of St. Lawrence cod stock has maintained genetic variability to sustain future evolution despite a dramatic population decline.

Résumé : À l'échelle globale, plusieurs stocks commerciaux de poissons ont connu des déclins spectaculaires à cause de la surpêche. De telles réductions démographiques dues à la pêche pourraient potentiellement diminuer la diversité génétique des populations de poissons marins. S'appuyant sur des analyses d'ADN faites sur des échantillons archivés et contemporains, notre étude compare la variabilité génétique à neuf locus microsatellites dans une population canadienne de morues franches (*Gadus morhua*) sur une période de 80 ans couvrant le temps d'avant l'intensification de la pêche jusqu'à maintenant alors que la population connaît une abondance historiquement basse. Des données génétiques solidement validées provenant d'échantillons étalés dans le temps ont servi à estimer la taille effective de la population. Durant cette période, nous n'observons de perte ni d'hétérozygotie, ni de diversité allélique. Plusieurs des méthodes d'estimation utilisées ne peuvent pas distinguer la taille effective de la population de l'infini et la limite inférieure de l'intervalle de confiance de 95 % des estimations est généralement >500, ce qui laisse croire que la taille effective est probablement beaucoup plus élevée que ce nombre. Il apparaît donc que le stock de morues du sud du golfe du Saint-Laurent a conservé la variabilité génétique nécessaire pour assurer son évolution future, malgré son déclin démographique spectaculaire.

[Traduit par la Rédaction]

Introduction

In recent years many commercial fish stocks have declined dramatically as a result of overfishing (Hutchings and Reynolds 2004; Worm et al. 2006). A growing body of literature has addressed the possible genetic consequences of such extensive fishing pressure in relation to selection on ecologically and demographically important traits such as growth and age and size at maturity (e.g., Stokes and Law 2000; Conover and Munch 2002; Law 2007). Meanwhile, there has generally been less focus on whether severe fisheries-induced population reductions have eroded the genetic diversity of exploited stocks.

The general lack of concern for this issue probably relates to the very large sizes of most exploited fish populations. Even after commercial collapse, these populations number millions of individuals. This is well above the population sizes typically considered at risk of losing genetic diversity (Frankham et al. 2002 and references therein). However, the rate of loss of genetic diversity is determined by the effective population size (N_e) rather than the census size (N), and several studies have suggested that the ratio between these

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two parameters (the N_e/N ratio) could be extremely low in marine fish compared with natural populations of most other organisms (see review by Hauser and Carvalho 2008). Surprisingly small effective population sizes of hundreds to a few thousand individuals have been reported for marine fish populations whose census sizes are up to five orders of magnitude larger (see e.g., Hauser et al. 2002; Hutchinson et al. 2003; Hoarau et al. 2005).

Although the degree to which such extreme estimates of N_e/N ratios reflect the true dynamics of biological systems has been questioned (Flowers et al. 2002; Poulsen et al. 2006), these findings caution that marine fish could be at greater risk of losing genetic diversity because of fisheries-induced population reductions than previously believed. Apparent loss of genetic diversity following intensive exploitation has already been reported for several marine fish species (Smith et al. 1991; Hauser et al. 2002; Hutchinson et al. 2003), but other studies have found temporal stability in genetic diversity despite heavy fishing pressure (Ruzzante et al. 2001; Jakobsdóttir et al. 2006; Poulsen et al. 2006). It is therefore important that we gain a better understanding of how vulnerable fish stocks are to erosion of genetic variation.

To this end, we studied temporal genetic variation in Atlantic cod (*Gadus morhua*) in the southern Gulf of St. Lawrence, Canada. This population has been exploited commercially for centuries, but fishing intensified in the 1940s, with landings peaking at >100 000 t in 1956 (Fisheries and Oceans Canada (DFO) 2008; Fig. 1*a*). Biomass and abundance of commercial-sized cod declined sharply in the 1960s and early 1970s, but then quickly increased again (because of a period of unusually strong recruitment) (Fig. 1*b*). However, like many of the other Canadian cod stocks, the southern Gulf of St. Lawrence stock suffered a second dramatic decline in the early 1990s, and at that time a moratorium on fishing was imposed because of the low abundance. While the collapse in this area was not at first as dramatic as in some of the nearby stocks (e.g., the northern cod off Newfoundland; Hutchings and Reynolds 2004), the population has not yet recovered despite more than 15 years of severely reduced fishing pressure. During the early population reduction in the 1960s, abundance declined less sharply for mature individuals than for the total or exploitable population (Fig. 1*b*). This is because the age at maturity declined markedly during this period (Swain 2010), so that increased spawning at young ages partly compensated for the loss of older spawners and the mean age of spawners substantially declined (Supplemental Fig. S1[1]). Thus, in addition to census size fluctuations, the population has undergone dramatic demographic changes, which could also affect the N_e, especially if reproductive success is correlated with body size. The estimated abundance of mature individuals is currently at the lowest level ever recorded. In 2008 the spawning stock was estimated to consist of about 44 million fish, about 8% of the recorded maximum and about one-third of the harmonic mean spawning stock size over the period 1950–2008 (118 million fish), and spawner abundance is expected to continue to decline (DFO 2008; Swain and Chouinard 2008).

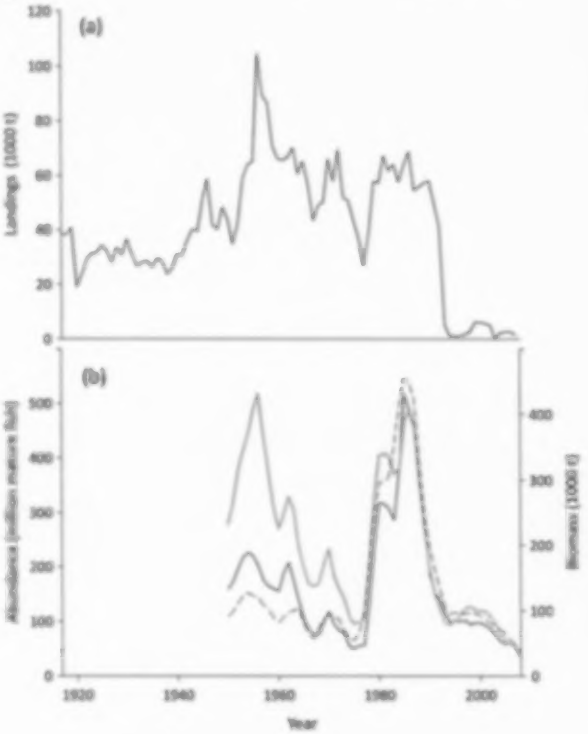


Fig. 1. Historical landings (*a*) and estimated biomass and abundance (*b*) for the southern Gulf of St. Lawrence cod stock. Panel *b* shows mature abundance (dashed line) as well as biomass (heavy grey line) and abundance (solid black line) of cod aged 5 years and older (the main ages contributing to the fishery). Landings and estimates of abundance and biomass at age are from Chouinard et al. (2008); mature abundance is computed based on revised maturity ogives estimated by D.P. Swain (unpublished data).

Based on DNA extracted from archived and contemporary samples, we compared the genetic composition of this population over a time span of 80 years from before the fishery intensified to the present. We conducted an extensive quality control of the historical data because the degraded DNA from these samples makes genotyping particularly error-prone. The results were used to investigate whether this population has suffered a loss of genetic diversity following intensive exploitation. To gain a better understanding of the observed pattern, we also applied several methods to estimate the N_e. With an estimated mean census size of 118 million individuals, we expect that the N_e of this population could still be large, and it is notoriously difficult to distinguish a large N_e from a very large one based on genetic data (Nei and Tajima 1981; Waples 1989; Palstra and Ruzzante 2008). For this reason — and because it arguably is the most relevant indicator for precautionary management — we focused particularly on the lower limits of the confidence intervals of N_e estimates to assess whether the N_e of this population could be of conservation concern.

[1] Supplementary data for this article are available on the journal Web site (http://cjfas.nrc.ca).

Material and methods

Samples

The source of historical DNA from the southern Gulf of St. Lawrence cod stock was a set of archived otoliths collected in 1928 near Paspébiac in the western end of the southern Gulf (Fig. 2). These otoliths (n = 57 individuals) were obtained from the National Institute of Aquatic Resources in Denmark and had been stored individually in paper envelopes at room temperature. Contemporary samples (n = 60 individuals) were obtained from gill tissue collected within <100 km of the historical sampling location from mature individuals at spawning time (May–June) in 2008.

Laboratory procedures

DNA was extracted from both historical and contemporary samples with an Omega EZNA Tissue DNA kit (Omega Bio-Tek, USA). This method was used because it has been demonstrated to perform well with otolith DNA samples and does not damage otoliths during extraction (Therkildsen et al. 2010). The gill tissue was processed according to the manufacturer's instructions. Otoliths were left in the digestion solution for 3 h and removed before DNA was purified from the extract following the same procedure as used for gill tissue.

The samples were genotyped at nine di-, tri-, and tetra-nucleotide microsatellite loci: *Gmo 2* (di-) and *Gmo 132* (di-) (Brooker et al. 1994); *Gmo 8* (tetra-), *Gmo 19* (tetra-), *Gmo 34* (tetra-), and *Gmo 35* (tri-) (Miller et al. 2000); *Tch 11* (tetra-) and *Tch 14* (tetra-) (O'Reilly et al. 2000); and *Gadm 1* (di-) (Hutchinson et al. 2001) (see Table 1 for allele size ranges). For the contemporary samples, polymerase chain reaction (PCR) amplification was conducted using 30 PCR cycles, while historical samples were amplified with 39 cycles. The PCR amplification products were analyzed on a Basestation51 automated sequencer (MJ Research/Bio-Rad), and allele sizes were scored using the CARTOGRAPHER Sequencing and Genotyping Analysis Software (MJ Research). Individuals with known genotypes were included in all gel runs to ensure correct fragment size scoring.

Quality control of the genotyping

Because DNA in historical samples is degraded and only available in small quantities, it is more prone to generating genotyping errors than DNA from contemporary samples (Taberlet et al. 1996; Pompanon et al. 2005). Therefore, several precautions were taken to minimize this risk. Both DNA extraction and PCR preparation with the historical samples were conducted in an isolated laboratory located in a separate building where no fish samples had previously been analyzed. Negative controls were used in all runs (both extraction and PCR). Furthermore, all historical samples were genotyped following a multiple tubes approach (Navidi et al. 1992; Taberlet et al. 1996) based on generating several independent replicates of each genotype, because consensus genotypes (consistent results from multiple reactions) should be more reliable than results from a single reaction.

Two amplifications were initially carried out for each individual at each locus. If the same genotype was obtained in both, this consensus genotype was recorded. If different genotypes were observed in the replicates, additional PCRs were conducted until each allele had been observed twice or a consensus homozygote genotype could be assigned. In cases where three or more replicate genotypes were identical but a single replicate was different from the others, we followed the methodology in Miller and Waits (2003) and devised the consensus genotype based on the principle of parsimony. Initial trials had shown that the two types of genotyping error — allelic dropout (non-amplification of one allele) and mistaken alleles (mismatch between assigned alleles and consensus genotype due to, e.g., PCR artifacts, mis-scoring, other human errors, or sporadic contamination) — were almost equally common. Hence, in an example of four replicates yielding genotypes AA, AA, AA, and AB, the most parsimonious consensus genotype would be a homozygote with one mistaken allele rather than a heterozygote where the same allele had dropped out three times. In cases where a consensus genotype could not be assigned after seven independent PCRs or where more than four amplification attempts had failed, the genotype was recorded as missing. Only consensus genotypes were used for further analyses. To assess the repeatability of results, >35% of genotypes (including all homozygotes at most loci) from the contemporary samples were also replicated in independent PCRs.

Genotyping error rates were assessed by recording mismatches between replicates and corresponding consensus genotypes (assuming the latter represented "true" genotypes). Allelic dropouts and mistaken alleles were recorded independently. We quantified errors both per amplified allele (as defined by Broquet and Petit (2004)) and the proportion of positive PCRs that contained at least one of the two error types (as suggested by Hoffman and Amos 2005; Pompanon et al. 2005). Finally, to identify genotyping errors not

Fig. 2. The southern Gulf of St. Lawrence, showing sampling locations for the 1928 sample (square) and the 2008 sample (circles). The 50, 100, and 200 m isobaths are indicated by the grey lines.

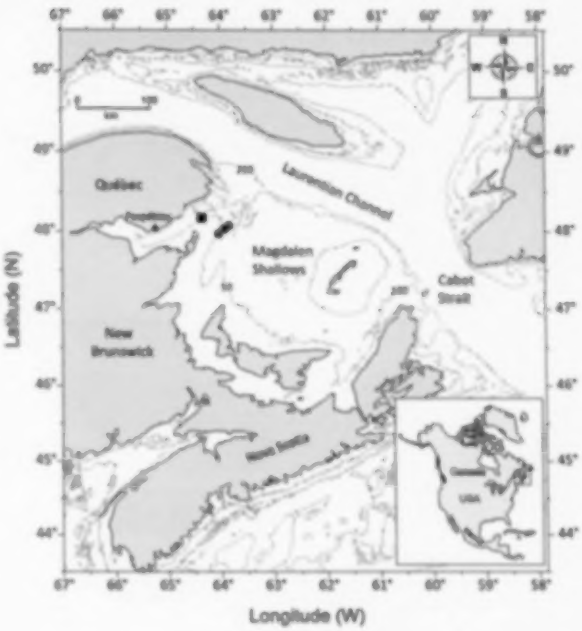


Table 1. Sample size (n), allelic richness (adjusted to 39 individuals), observed (H_o) and expected (H_e) heterozygosity, p value for the tests of deviation from Hardy–Weinberg Equilibrium (HWE), and locus-specific F_{ST} for the two temporally spaced samples from southern Gulf of St. Lawrence cod.

		n		Allelic richness		H_o		H_e		p (HWE)		
Locus	Size range (bp)	1928	2008	1928	2008	1928	2008	1928	2008	1928	2008	F_{ST}
Gmo 2	107–153	46	60	12.5	12.3	0.87	0.77	0.83	0.81	0.44	0.26	0.005
Gmo 8	121–289	46	60	13.7	16.6	0.89	0.88	0.92	0.92	0.13	0.84	0.001
Gmo 19	120–224	40	60	20.9	19.0	0.98	0.95	0.94	0.94	0.86	0.19	0.000
Gmo 34	095–111	49	60	4.8	4.6	0.65	0.43	0.57	0.43	0.93	0.16	0.016
Gmo 35	121–148	47	60	7.6	7.6	0.79	0.73	0.77	0.78	0.35	0.44	0.005
Gmo 132	111–125	47	60	6.0	6.5	0.79	0.58	0.70	0.63	0.67	0.17	0.009
Tch 11	115–215	39	60	18.0	20.0	0.92	0.92	0.93	0.94	0.32	0.54	0.001
Tch 14	103–231	45	60	19.3	19.3	0.96	0.87	0.94	0.93	0.14	0.42	–0.008
Gadm 1	168–180	45	60	5.9	5.6	0.62	0.55	0.62	0.59	0.29	0.21	–0.008
Average across loci		45	60	12.1	12.4	0.83	0.74	0.80	0.77	0.27*	0.25*	0.002*

*Results from multi-locus test or estimation.

detectable through replication (see DeWoody et al. 2006), the program MICRO-CHECKER (Van Oosterhout et al. 2004) was used to test for evidence of stutter, null alleles, and large allele dropout in the consensus data sets for both 1928 and 2008.

Testing assumptions for the statistical analysis

Deviations from Hardy–Weinberg proportions for each locus and sample as well as genotypic disequilibrium between all pairs of loci in each sample were tested using the program FSTAT version 2.9.3.2 (Goudet 1995, 2001). A sequential Bonferroni correction was applied to correct for multiple tests (Rice 1989). In addition, to test for evidence of selection effects on any locus, a neutrality test was applied using the Bayesian method implemented in the software BAYESCAN (Foll and Gaggiotti 2008).

Comparison of genetic diversity

Using FSTAT version 2.9.3.2 (Goudet 1995, 2001), the genetic diversity of each sample was quantified by Nei's (1987) unbiased heterozygosity and by allelic richness (adjusted to 39 individuals, which was the smallest number of individuals genotyped for any locus; El Mousadik and Petit 1996). To test for differences in diversity between samples, these statistics were computed for each locus in each sample and were compared by a Wilcoxon's signed rank test. We used the χ^2 test implemented in the program CHIFISH (Ryman 2006) to test for differences in allele frequency distributions at any locus between the samples. The genetic differentiation between samples was also estimated by single- and multi-locus F_{ST} values following Weir and Cockerham (1984). The 95% confidence interval on the multilocus estimate was determined by bootstrapping over loci, and the significance was tested by permuting genotypes between samples using the test procedure by Goudet et al. (1996) and 1000 permutations as implemented in FSTAT version 2.9.3.2 (Goudet 1995, 2001).

Estimation of the effective population size: single sample estimator

We applied the linkage disequilibrium (LD) method to obtain separate estimates of N_e in 1928 and in 2008. This method is based on the notion that LD at neutral, unlinked loci in isolated randomly mating populations should arise only as a result of drift, and hence the level of nonrandom association of alleles at different loci should reflect the N_e of the population (Waples 2006; Waples and Do 2009). We estimated N_e with each sample using the program LDNE (Waples and Do 2008). Since allele frequencies close to 0 or 1 can affect this method in ways that are not completely understood (Waples 2006), we used a cutoff point at frequencies >0.02 (excluding single copy alleles), which has been shown to minimize bias for simulated data sets similar to ours (Waples and Do 2009). Estimates of N_e were computed based on the entire data set, but to assess sample size effects when comparing the 2008 results with the 1928 sample, the analysis was repeated on a subset of 40 randomly selected individuals from 2008.

Estimation of the effective population size: the temporal method

The temporal method for estimating N_e is based on quantifying the amount of drift or the rate of coalescence that has occurred between two or more samples taken at different times from the same population (Leberg 2005; Wang 2005). No universally superior estimator has been identified (Tallmon et al. 2004; Jorde and Ryman 2007), so we compared several different applications that are based on different mathematical properties. Common to these approaches is the assumption that mutation, migration, and selection have not significantly influenced the variance in allele frequencies between samples.

Estimating the generation length

Application of the temporal method to estimate N_e requires information about the number of generations between samples. The generation length of populations with overlapping generations is equal to the mean age of parents (Hill 1979). Following Miller and Kapuscinski (1997), this was approximated as the mean age of spawners weighted by age-specific fecundity. The relative fecundity of each female age class was calculated based on the stock-specific relationship between age and fecundity (McIntyre and Hutchings 2003). Abundance and maturity data on each age class (D.P. Swain, unpublished data) were then weighted by these relative fecundities. Although differences in reproductive

success among males have been demonstrated in captivity (Bekkevold et al. 2002), there are no data to quantify this effect in the wild. Therefore male reproductive contribution was assumed to be equal across age classes.

An average of the weighted female and unweighted male age distributions of spawners was computed for each year in the period 1950–2008 (Supplemental Fig. S1[2]). The overall average generation time in the period was 6.35 years, corresponding to 12.6 generations between the samples. This is probably a slight overestimate because the average age of spawners has declined during the period (Swain 2010; Supplemental Fig. S1[2]), and no data were available for 1928–1950 when it was likely to have been higher. Hence, for the analyses with the temporal method, we applied an estimated span of 12 generations. To assess the sensitivity of our N_e estimates to uncertainty in generation time, all analyses were also conducted assuming spans of 9 and 15 generations.

Moment estimator

The classical estimator of Waples (1989) is widely applied for estimating temporal N_e. However, Jorde and Ryman (2007) developed a modified, less biased moment estimator for small sample sizes and skewed allele frequencies (many rare alleles), as characterized in this data set. We used the program TEMPOFS (Jorde and Ryman 2007) to estimate N_e with this modified estimator.

Pseudo-likelihood estimator

In addition to the computationally simple moment estimator, we also used the pseudo-likelihood method of Wang (2001) as implemented in the program MNE 2 (Wang and Whitlock 2003). This method finds the value of N_e that maximizes the probability of the data based on a Wright–Fisher model of genetic drift and uses more information about allele frequencies in the samples than do moment-based estimators. The program requires an input for the maximum value that N_e may assume (N_{eMAX}), and we conducted separate analyses with an N_{eMAX} of 10^3, 10^4, and 3×10^4 (the largest value the program could handle on a standard computer).

ABC estimator

As a final application of the temporal method, N_e was estimated with approximate Bayesian computation (ABC) implemented in the program DIY ABC (Cornuet et al. 2008). Based on summary statistics known to have a relationship with N_e, this method compares the observed data set sampled from the studied population with an unknown N_e against 500 000 data sets sampled temporally from simulated populations, each with a known N_e. We used different sets of broad uniform priors on N_e ranging from 1 to 10^6 and a generalized stepwise mutation model with the prior parameters used in Cornuet et al. (2008). The posterior distribution for N_e was generated with a local logistic regression based on logit-transformed estimates of N_e from 5000 simulated data sets (the 1% most similar to the observed data). Three independent runs were conducted for each set of priors for N_e to check the consistency of results, and the presented results are averages of the mode and the 0.025 and 0.975 quartiles between these three runs.

Effect of genotyping error

To assess the potential effect of genotyping errors without quality control on our N_e estimates, all analyses were repeated with an uncorrected data set consisting of the first genotypes observed for each individual at each locus.

Results

Genotyping

Of the 57 historical individuals, eight were removed in a prescreening: three because they showed signs of multiple genotypes (pre-extraction sample contamination) and five because of poor amplification. For the remainder of the sample, on average 3.1 (range 2–7) positive PCRs were genotyped per locus per individual. The number of consensus genotypes obtained for the historical samples ranged from 39 to 49 per locus (Table 1). In single reactions, 7.9% of amplified alleles were classified as mistaken, and the average rate of allelic dropout was 8.9% (Supplemental Table S1[2]). The amplification success was higher for loci with shorter allele sizes (Supplemental Fig. S2[2]); thus, large allele dropout may have affected our historical data. Indeed, for six of the nine loci, the largest allele dropped out more often (data not shown). The effect of this on the consensus data appeared to be limited, however, as no significant differences in average allele size or allelic richness between the 1928 and the 2008 sample were detected (see below). Likewise, tests conducted in MICRO-CHECKER on the consensus data set did not detect evidence of large allele dropout, stutter, or null alleles.

The overall reliability of the consensus data can be coarsely approximated by calculating the probability of assigning a wrong allele to the consensus genotype after two independent reactions (i.e., the same error occurring in both replicates) as the squared probability of each type of error. Since average rates of mistaken alleles and allelic dropout were both around 8%–9%, this error is $\approx 0.8\%$. With additional replication (>2) for many genotypes, the actual error rate is likely even lower. Hence, despite the relatively high error rate for single amplifications, the overall reliability of the consensus data is likely above 99%.

All of the 60 contemporary samples were successfully genotyped at all loci. The replication of a subset of genotypes revealed an allelic dropout rate of 0.9% and 0.5% mistaken alleles, but these were corrected through replication.

Testing assumptions for the statistical analysis

The data showed no significant deviations from Hardy–Weinberg expectations for any locus in any sample (Table 1), and none of the pairwise comparisons in the linkage disequilibrium test were significant after Bonferroni correction. The neutrality test did not detect any outlier loci; the posterior probability of being affected by selection was ≤0.62 for all loci, and the results were consistent between independent runs (results not shown). Hence all loci were included in further analyses.

Comparison of genetic diversity

The average allelic richness was slightly higher in 2008 than in 1928, while the average expected heterozygosity was slightly higher in 1928 (Table 1). However, none of

these differences were significant on a locus-by-locus basis ($p > 0.17$ for both). There were also no significant differences in allele frequencies between the samples ($p = 0.51$), and the multilocus F_{ST} estimate was 0.002 (95% confidence interval: –0.002 to 0.006) and not significantly different from zero ($p = 0.48$).

Estimation of the effective population size

The results obtained with the different N_e estimators are summarized (Fig. 3). With the LD method, the point estimate for 1928 could not be distinguished from infinity. For 2008, the method yielded a finite estimate of 580 individuals (895 when the analysis was based on only 40 individuals), but the 95% confidence interval included infinity. While the point estimate for 2008 is smaller than that for 1928, the confidence intervals broadly overlap (even extending slightly lower in 2008 than in 1928; see Fig. 3), making the data inconclusive about possible changes in N_e over time. In both years, the lower bound of the confidence interval was around a few hundred.

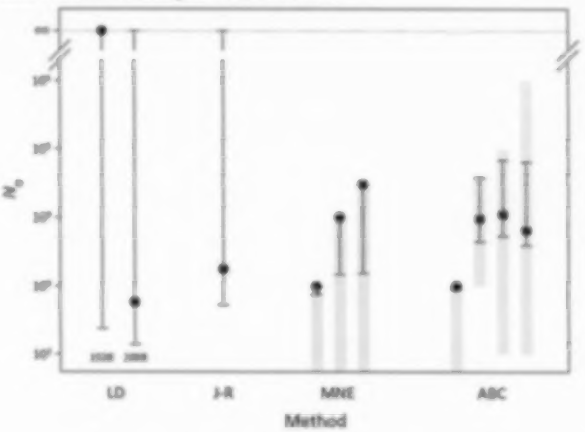
All three applications of the temporal method yielded somewhat larger point estimates. The Jorde–Ryman moment estimator provided a finite point estimate for N_e of 1768 with 95% confidence intervals spanning from just over 500 to infinity. The pseudo-likelihood-based estimator generated point estimates that were equal to or very close to the maximum allowed for all values of N_{eMAX} tested (Fig. 3), suggesting that N_e is $>3 \times 10^4$ (which was the largest size that could be tested here). The lower 95% confidence limit, however, was consistent between runs at around 10^3 regardless of the N_{eMAX}. The ABC method provided fairly consistent estimates of N_e for a broad range of prior values (Fig. 3), and it was the only method that yielded finite confidence intervals that were not constrained by the priors. When the maximum N_e allowed was $<10^4$ (the estimation process was limited to within the interval given in the priors), the estimate was exactly the maximum value, suggesting that N_e was larger than this. For all other prior sets tested, the estimate of N_e was around 10^4, and lower and upper 95% confidence limits were around 5×10^3 to 6×10^4, respectively. Results were similar for independent runs with the same priors.

Varying the assumed number of generations between samples affected the Jorde–Ryman and the MNE estimator in predictable ways. Reducing the span to nine generations caused a ~25% decrease in the lower confidence interval limit, while increasing it 15 generations caused a corresponding ~25% increase. The results obtained with the ABC method were more variable, but with lower confidence interval limits only changing by up to ~10%. Based on available data for this population (Supplemental Fig. S1[2]), spans of 9 and 15 generations over the 80-year period appear to represent the extremes of plausible values. Thus these effects represent the maximum bias expected from uncertainty in estimating this parameter.

Effects of genotyping error

Analysis of the uncorrected data revealed large deviations from Hardy–Weinberg equilibrium (HWE) proportions at several loci in the 1928 sample and significant differences in allele frequencies between the two samples ($p = 0.03$). The average heterozygosity in both samples was identical to that computed for the consensus data set, while the average allelic richness was slightly lower both for the 1928 and the 2008 sample in the uncorrected data, though still not significantly different between samples ($p = 0.20$). The LD estimate based only on the 2008 sample was slightly higher with the uncorrected data, but all N_e estimates involving the historical sample (which was particularly error prone) were considerably lower with the uncorrected data (Supplemental Fig. S3[2]). Point estimates were at least 50% lower, and lower confidence interval limits were at least 35% lower than those obtained with the consensus data. The two methods for which point estimates were beyond the estimation capabilities with the consensus data (LD and MNE) also yielded finite estimates with uncorrected data (Supplemental Fig. S3[2]).

Fig. 3. Point estimates (dots) and precision limits (error bars) of N_e obtained with different estimators. LD is the linkage disequilibrium method applied separately to the 1928 and the 2008 sample. J–R is the Jorde–Ryman temporal method, MNE is the pseudo-likelihood method, and ABC is the method based on approximate Bayesian computation. Error bars represent 95% confidence intervals (2.5% and 97.5% quartiles of the posterior distribution for the ABC). LD and J–R are moment-based and incorporate no prior. MNE uses a uniform prior from 0 to N_{eMAX}, and the ABC estimates are based on uniform priors from N_{eMIN} to N_{eMAX}. The priors used in different runs are illustrated by the shaded bars.



Discussion

Although a large reduction in census population size inevitably will result in the loss of many rare alleles at the genomic level (Ryman et al. 1995; Allendorf et al. 2008), our study suggests that the southern Gulf of St. Lawrence cod stock has not suffered any reduction in common genetic diversity indices at microsatellite loci despite severe population reductions due to intensive fishing over the past 80 years. The selection of methods applied to estimate the N_e varied in their point estimates and their precision, but generally had overlapping confidence intervals. The LD estimator suggested that N_e could be as low as a few hundred, but with a point estimate of infinity for the 1928 sample and exceedingly wide confidence intervals, this was also the most imprecise and uninformative estimator. All applications of the temporal method (based on more data using

both samples) suggested that N_e was larger than 500–5000 (lower 95% confidence interval limit). The consistent exclusion of very low values of N_e from the confidence intervals by these three methods that are based on different models with varying assumptions and different mathematical approaches provides compelling evidence that the N_e of this population is at the very least 500 and most likely considerably larger than that. This finding is consistent with the observation of temporal stability in genetic diversity because an N_e much above 500 exceeds the population sizes typically associated with immediate loss of genetic diversity due to drift (Frankham et al. 2002 and references therein).

Precision of estimates

The study remains inconclusive about the exact magnitude of N_e. The LD method was uninformative, with confidence intervals extending from a couple of hundred individuals to infinity. The point estimates from the temporal method indicated that it was either 2000 – 10 000 (Jorde–Ryman and ABC) or considerably greater (MNE). This uncertainty is not surprising given that in general, genetic N_e estimators do not perform well for large populations (Nei and Tajima 1981; Waples 1989) because when there is little drift, the signal-to-noise ratio becomes very small. This may compromise the accuracy of all the estimators, and it is not obvious which methods would perform best under such circumstances, as comparative simulation studies are typically only carried out for small to moderate N_e (see e.g., Tallmon et al. 2004; Jorde and Ryman 2007). In general, however, there are several ways that the precision of our estimates could have been improved: (*i*) increasing the number of individuals in each sample; (*ii*) increasing the number of generations between samples; (*iii*) integrating information from additional temporal samples; or (*iv*) increasing the number of loci or the variability of the loci used (Waples 1989; Wang 2001; Palstra and Ruzzante 2008).

Recent papers have emphasized that a very extensive sampling of individuals may be needed to achieve estimates with finite bounds when N_e is large (Ovenden et al. 2007; Palstra and Ruzzante 2008), but studies based on archived samples are limited by the number of historical individuals available and the extra time and resources required for their reliable genotyping. Although the sample size used in this study for this reason was modest, it is typical of studies using temporal sampling to estimate N_e (see Palstra and Ruzzante 2008). The finding of no genetic differentiation between temporal samples (with a narrow confidence interval of the F_{ST} estimate) also suggests that sampling effects were low. Reducing these sampling effects further (by increasing sample size) would thus likely have lead to even smaller estimates of variance in allele frequencies, and hence larger sample sizes for this study could possibly have increased N_e estimates and narrowed confidence limits, but would unlikely have reduced the lower limit.

The relatively small sample size was also compensated for by using the oldest available samples providing the maximum number of generations between samples possible. With extensive resources for genotyping, multiple sampling times could have improved precision and accuracy and allowed inference about potential temporal changes in the N_e. However, if only a limited number of individuals can be genotyped, information content is maximized by pursuing temporal extreme samples rather than spreading out the sampling within the time period studied.

While adding more loci may be the most straightforward way of increasing the power for N_e estimation, we expect that the number of loci analyzed would need to be considerably increased to achieve a markedly greater precision. Since we found no significant changes in allele frequencies at any of the loci studied, additional neutral loci are unlikely to show a significantly elevated drift signal. When this is the case, the estimators hardly have any temporal variance to work with — even with vast numbers of loci or samples. Accordingly, adding a few more loci is unlikely to add much power. A many-fold increase in the number of loci used may improve the statistical precision, but as pointed out by Palstra and Ruzzante (2008), the practical value of obtaining precise estimates of N_e when it is large may not always justify the extensive efforts required. While it may be of general interest to derive reliable inference about the N_e/N ratio in large populations, the main question of interest to management is whether N_e is small. It has been suggested that an N_e of up to 5000 may be required for maintaining evolutionary potential in the long term (e.g., Lande 1995). The ABC method indicated that N_e for the southern Gulf of St. Lawrence cod exceeded this value, but it was not excluded from the confidence intervals obtained with other methods. However, our estimates are in general sufficiently precise to demonstrate that the N_e of this population is unlikely to be below the more commonly considered critical size of 500 individuals (Frankham et al. 2002).

Comparison to N_e estimates in other studies

The N_e estimates presented here are similar in magnitude to those presented in several other studies on marine fish (e.g., Hoarau et al. 2005; Saillant and Gold 2006), but are much larger than the extremely small values of N_e of a few hundred that have been proposed for a North Sea cod population (Hutchinson et al. 2003) and for a New Zealand snapper stock (*Pagrus auratus*) (Hauser et al. 2002). One possible reason for the higher N_e estimates obtained in this study could simply be that the census size of the population is more than an order of magnitude larger than in the cited studies. If there were a fairly constant N_e/N ratio for marine fish populations, we would expect a proportionately larger effective size. However, the N_e/N ratio is likely to vary between populations depending on both environmental conditions and variation in life history parameters, so the discrepancies could reflect real population-specific differences. Nevertheless, it is also possible that the results presented here overestimate the true N_e or that results from other studies represent underestimates, as there are a range of factors that can affect N_e estimates, including violation of model assumptions and technical problems (e.g., through unidentified population structure, migration, genotyping errors, and inadequate sampling of individuals and loci).

Model assumptions

The temporal method is based on a model with discrete generations and assumes that mutation, selection, and migration are unimportant in changing population allelic frequencies relative to genetic drift. The assumption of discrete

generations is obviously violated because cod populations have overlapping generations. Using empirical data from the literature, Palstra and Ruzzante (2008) have confirmed that ignored age structure has probably introduced a strong bias into many empirical N_e estimates. However, simulations have shown that with about 12 generations between samples (as was the case here), the bias induced by this violation is negligible (Waples and Yokota 2007). The number of generations is probably also sufficiently low for effects of mutations to be safely ignored (Waples 1989; Beaumont 2003). However, the assumptions of no effects of selection on the markers used and of complete isolation of the studied population may be more problematic.

Microsatellites are generally expected to be neutral markers, and we did not detect evidence of temporal selection on any locus over the time span studied here. However, the power of this test was low because of the relatively small number of loci and samples (Guinand et al. 2004), and other studies have shown that two of the loci we employed (*Gmo 34* and *Gmo 132*) may be affected by selection (Nielsen et al. 2006; Westgaard and Fevolden 2007). In fact, these two loci showed the highest F_{ST} differentiation between samples, and although the changes in allele frequencies were not significant, the slightly larger differentiation could possibly indicate directional selection affecting these loci. This, in turn, generates larger variance in allele frequencies, resulting in smaller N_e estimates. Excluding *Gmo 34* and *Gmo 132* from analyses yielded point estimates and lower confidence interval limits 10%–65% higher than those based on all loci. Thus, including these two loci in our study could have biased the N_e estimates downwards, making our results conservative in terms of evaluating whether N_e could be small. Point estimates and lower confidence limits may for this reason be substantially larger than reported here.

In relation to migration, there are probably few completely isolated populations in nature, and this is especially true for marine fish that have long dispersal capabilities and live in an environment with few strong physical barriers to gene flow. Hence, the question is not whether the assumption of no gene flow is violated, but rather whether the effect is sufficiently small to safely be ignored. Migration can cause either an under- or an over-estimation of N_e depending on the degree of differentiation from source populations and the time scale involved (Wang and Whitlock 2003; Fraser et al. 2007). Migration from a dissimilar source can increase the change in allele frequencies between two temporally spaced samples, and if this is interpreted as a higher level of drift it will be translated to an underestimate of the N_e. Migration from a genetically similar source, on the other hand, may counteract the effects of drift by homogenizing gene frequencies between sampling times, and in such cases estimates of N_e could reflect the size of a larger metapopulation rather than the local population sampled (Wang and Whitlock 2003).

Given that this study detected virtually no signal of drift, only the latter scenario may be of concern. Based on the variation at six hypervariable microsatellites, Ruzzante et al. (2000) found that there was a significant genetic distance between cod samples within the Gulf of St. Lawrence and cod sampled from different nominal stocks in its approaches. However, they did not find significant differentiation between the two nominal stocks within the Gulf, the southern and the northern stocks. Based on the available evidence, it cannot be excluded that gene flow from the northern Gulf has caused an overestimate of the effective size of the population residing in the southern Gulf.

However, both traditional tags and otolith elemental fingerprints as natural tags suggest a high degree of reproductive isolation between the cod in the northern and southern Gulf (Campana et al. 2000; Robichaud and Rose 2004). Further, there are significant differences in vertebral counts between the northern and southern populations (Swain et al. 2001). While this morphological trait is affected by environmental factors, it also has a genetic component and has been proposed to be fitness-related in other species (Billerbeck et al. 1997; Swain and Foote 1999). The difference may thus represent adaptive divergence between the populations. Such divergence was recently found between southern Gulf of St. Lawrence cod and other nearby populations, as there were genetically based differences in life history traits over scales with little differentiation at microsatellite loci (Hutchings et al. 2007). Therefore, the absence of differentiation at neutral markers between the southern and northern Gulf cod may reflect historical events and large effective population size rather than contemporary gene flow.

Undetected population substructure within the southern Gulf could also affect the spatial scale for which an N_e estimate applies. There is currently no genetic evidence available to suggest population structure within the southern Gulf itself, but should such structure exist, our N_e estimates would apply only to a portion of the nominal stock and hence underestimate the magnitude for the whole area.

Genotyping errors

In addition to violation of model assumptions, genotyping errors can also affect the N_e estimates. While probably few data sets on genetic markers are error free, there is a particular concern when working with historical material (Hoffman and Amos 2005; Pompanon et al. 2005). As demonstrated by our estimates based on uncorrected data, genotyping errors in the historical sample could lead to inflated measures of differentiation between temporal samples and hence marked underestimates of N_e. Despite our large efforts to minimize this effect, residual errors in our data set (arising from large allele dropout or other) may have biased our reported estimates downward. However, such errors are likely to cause departure from HWE, create signs of LD between loci, or cause divergence between samples, and none of these phenomena were observed. In combination with the failure of the software MICRO-CHECKER to detect deviations from expected frequency distributions of allele sizes and genotypes, this strongly suggests that genotyping error did not significantly influence our results.

Quantification of genotyping errors and incorporation of special precautions to mitigate their effect are standard procedures in most population genetic studies based on noninvasive samples (hair, feces, urine, etc.; Taberlet et al. 1999; Broquet and Petit 2004) and have been widely applied in studies using museum specimens as a source of DNA (e.g., Miller and Waits 2003; Sefc et al. 2003; Frantz et al. 2008). However, this issue is often not explicitly discussed in his-

torical genetic studies based on archived fish scales and otoliths. This is somewhat surprising given that this source of historical DNA may be available in smaller quantities and may be more susceptible to pre-extraction contamination than other sources of historical DNA, such as bone or teeth (because DNA is recovered from the surface of otoliths and hence samples cannot be decontaminated prior to extraction). The present study clearly demonstrates that genotyping errors, if not accounted for, can pose considerable problems when working with these kinds of samples. We therefore highly encourage explicit quantification of genotyping error rates and careful quality control of data in future studies with historical otolith and scale samples.

Implications

Given that in the current study, the genetic data were extensively validated and the assumptions of the N_e estimation methods appear to be met, our findings clearly suggest that the cod population in the southern Gulf of St. Lawrence has a large effective population size and has not suffered any loss of heterozygosity and allelic richness at neutral markers over the past 80 years. This suggests that the extremely small estimates of N_e reported in other studies do not appear to be a universal phenomenon for marine fish populations.

The large N_e of the southern Gulf of St. Lawrence cod stock implies that future evolution in this population is unlikely to be constrained by reduced genetic variability. It also implies, however, that selection will be an effective force in driving this future evolution, as selection is counteracted by drift to a lesser extent in large populations than in small ones (Frankham et al. 2002 and references therein). Considering that the N_e estimates obtained here may be greater than estimates reported for many other populations of marine fish, southern Gulf of St. Lawrence cod may be particularly vulnerable to effects of fisheries-induced selection (as well as other contemporary selection pressures), which may reduce the capacity of the population to demographically deal with environmental perturbations. The data presented here on temporal stability at neutral markers will serve as an important baseline for future evaluations of adaptive evolution at the DNA level.

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Catastrophic disturbances in headwater streams: the long-term ecological effects of debris flows and debris floods in the Klamath Mountains, northern California

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Abstract: Debris flows and debris floods are catastrophic disturbances in steep, mountainous landscapes throughout the world, but little is known about the long-term ecological effects of these events on headwater streams. In 10 basins (10–20 km^2) in the Klamath Mountains, northern California, USA, we used a space-for-time substitution to infer the long-term (10–100 years) effects of debris flows on stream ecosystem structure. Debris flows mobilized sediment and wood and removed riparian vegetation from large portions of channel networks. Stream temperatures were significantly warmer in streams that had recent debris flows (<10 years ago). Large wood, benthic organic matter, and detritivorous stoneflies were all very sparse in recent debris flow streams, suggesting that allochthonous energy pathways took decades to recover. Rainbow trout (*Oncorhynchus mykiss*) were abundant in recent debris flow streams, but populations of other vertebrates such as coastal giant salamander (*Dicamptodon tenebrosus*) and coastal tailed frog (*Ascaphus truei*) were virtually absent. Increased frequencies of catastrophic debris flows associated with forest management practices can have significant negative impacts on aquatic biodiversity in forested, mountainous landscapes.

Résumé : Les coulées et les crues de débris constituent des perturbations catastrophiques dans les paysages escarpés et montagneux partout dans le monde; on connaît, cependant, peu de choses sur les effets à long terme de ces événements sur les cours d'eau d'amont. Dans 10 bassins versants (10–20 km^2) dans les monts Klamath, nord de la Californie, É.-U., nous avons utilisé la substitution du temps par l'espace pour déduire les effets à long terme (10–100 ans) des coulées de débris sur la structure des écosystèmes d'eau courante. Les coulées de débris mobilisent les sédiments et le bois et arrachent la végétation riveraine sur une grande partie des réseaux de chenaux. Les températures de l'eau sont significativement plus élevées dans les cours d'eau qui ont connu des coulées de débris récentes (< de 10 ans). Les grands débris ligneux, la matière organique benthique et les plécoptères détritivores sont tous très réduits dans les cours d'eau qui ont subi récemment des coulées de débris, ce qui laisse croire que la voie énergétique allochtone met des décennies à se rétablir. Les truites arc-en-ciel (*Oncorhynchus mykiss*) sont abondantes dans les cours d'eau affectés récemment par des coulées de débris, mais les populations des autres vertébrés, tels que la grande salamandre du nord (*Dicamptodon tenebrosus*) et la grenouille à queue côtière (*Ascaphus truei*), sont pratiquement absentes. Les fréquences accrues des coulées de débris catastrophiques associées aux pratiques forestières peuvent avoir des impacts négatifs significatifs sur la biodiversité aquatique dans les paysages forestiers de montagne.

[Traduit par la Rédaction]

Introduction

Headwater streams are tremendously complex and dynamic ecosystems that connect terrestrial and aquatic environments (Gomi et al. 2002; Richardson and Danehy 2007). Water, sediment, and wood are routed from hillslopes into headwater streams during rare, episodic processes (Benda et al. 2005; May 2007). Headwater streams are also closely coupled with surrounding terrestrial ecosystems by various energy pathways, resulting in complex food web linkages and cross-habitat subsidies (Nakano and Murakami 2001; Baxter et al. 2005). As a consequence of these reciprocal fluxes, headwater streams can be greatly affected by disturbances in terrestrial ecosystems such as forest harvest (Gomi et al. 2002). Although steep headwater streams can make up a large portion of the total channel length of mountain stream networks (e.g., 50%–90%; Stock and Dietrich 2003;

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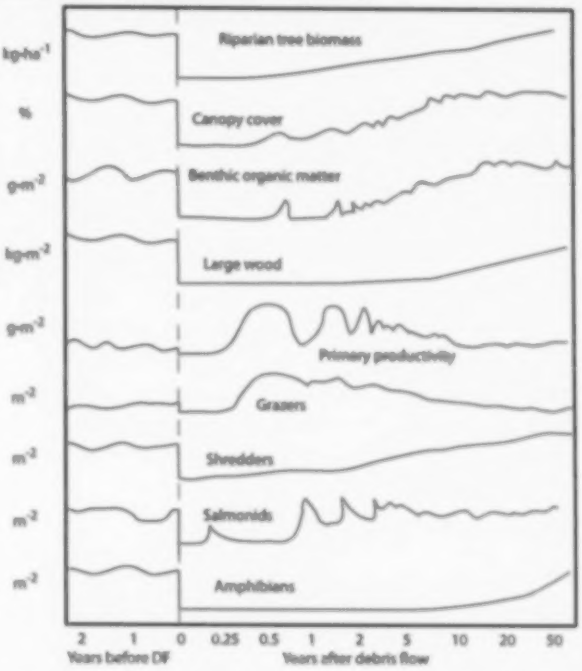
Benda et al. 2005), the ecosystem dynamics and ecosystem services provided by headwater streams remain poorly understood and are often underappreciated (Richardson and Danehy 2007).

Although lower gradient streams experience roughly annual fluvial disturbances, rare and catastrophic disturbances are the norm in mountainous headwater systems (Montgomery and Buffington 1997). Debris flows are a fundamental control on the morphology and habitat features of steep, headwater streams in the Pacific coastal ecoregion of North America (e.g., Montgomery et al. 2003; May 2007) and in mountainous landscapes around the world (Eaton et al. 2003; Stock and Dietrich 2003). Channel slope is the primary factor controlling the occurrence of the debris flows: channels steeper than 6%–10% are susceptible to debris flow scour or transport processes (Benda and Cundy 1990; Stock and Dietrich 2003). As described in this paper, debris flows in steep landscapes can travel for many kilometres down third-order streams with drainage areas >10 km^2, streams not normally thought of as "headwaters" (Benda et al. 2005; May 2007). Because they are relatively infrequent and rarely observed, the long-term geomorphic and ecological consequences of debris flows in headwater streams may be underappreciated.

Although the geomorphic effects of debris flows on stream channels have received considerable attention (e.g., Montgomery et al. 2003; Stock and Dietrich 2003), less is known about the effects of debris flows on stream ecosystem structure and food web dynamics. Several case studies have examined the short- or medium-term (0–6 years) impacts of debris flows on stream ecosystems (Lamberti et al. 1991; Kiffney et al. 2004; Snyder and Johnson 2006). Based on these case studies, as well as research on the effects of catastrophic watershed disturbances such as fire (e.g., Minshall et al. 1989), we propose several hypotheses for the medium- to long-term responses of stream ecosystems in the mountainous Pacific coastal ecoregion to debris flow disturbances (Fig. 1). The removal of macroscopic life in the stream and immediate riparian habitats by debris flows would result in immediate and long-lasting changes to ecosystems (Lamberti et al. 1991). Following debris flows, fast-growing, pioneer tree species such as willow and alder would re-establish fairly quickly, but biomass of riparian vegetation would remain low for many decades until late-successional conifers became well established (Fig. 1). Debris flows would remove the vast majority of large wood (LW) from affected reaches, and substantial recruitment of large-diameter wood would not occur for 50–100 years. Similarly, stores of benthic organic matter would be severely reduced and recovery of detrital stores would take at least a decade because retention of leaves would initially be low (Fig. 1). With the establishment of pioneer tree species, canopy cover would return to near predisturbance levels within 5–10 years (Fig. 1). Increased sunlight in the years following debris flow scour would result in the primacy of autochthonous energy pathways; therefore, high levels of primary production by algae would allow for large populations of grazing consumers (Lamberti et al. 1991; Kiffney et al. 2004). However, stream food webs would undergo a gradual shift from autochthonous to allochthonous-dominated energy sources as the forest canopy gradually

Fig. 1. Hypothetical responses of ecological parameters in a stream in the Pacific coastal ecoregion to a debris flow event. The vertical broken line indicates the occurrence of the debris flow (DF). Note that the *x* axis is approximately logarithmic.



shades the stream (Fig. 1). These shifts in productivity and food quality would undoubtedly affect the composition of primary consumers, which would have cascading effects on higher trophic levels such as vertebrate predators (Bilby and Bisson 1992; McNeely et al. 2007). For example, the increased productivity associated with open canopies could result in increased populations and growth rates of fish, as has been reported in experimental studies (Wilzbach et al. 2005). We hypothesize that aquatic obligate species would experience local extirpations if debris flows affected large portions of the channel network of headwater basins. Animals that are migratory (e.g., fish) or have aerial dispersal (e.g., most aquatic insects) have been found to recolonize fairly quickly following debris flows (Lamberti et al. 1991; Roghair et al. 2002; Kiffney et al. 2004), but less mobile animals such as amphibians could require many years to recolonize denuded headwater streams from downstream sources or across drainage divides.

In this study, we assess the long-term effects of debris flows on stream ecosystem structure and food web dynamics. For obvious reasons, replicated, long-term studies of these rare disturbance events are difficult to conduct. Instead, we used a space-for-time substitution to examine stream ecosystem structure in 10 streams that had debris flows at various times in the past. Background rates of debris flows in individual basins are quite low (recurrence intervals of 100–1000 years), but forest management practices can result in increased frequencies of debris flows, with important downstream effects on stream ecosystems (e.g., Montgomery et al. 2000; May 2002). The motivation for

Table 1. Geomorphic characteristics of study reaches.

Stream	Site code	Basin area (km^2)	Slope (%)	Bankfull width (m)	Valley width (m)	D50 grain size (mm)	Altered channels (%)		
							1964	1974	1997
Middle	Mid1997	18.1	9.3	5.1	21	272	38	26	31
Portuguese	Por1997	16.3	7.0	4.9	11	107	34	10	23
Rancheria	Ran1997	17.8	5.6	5.2	24	90	58	16	55
Tompkins	Tom1997	21.0	5.3	4.3	43	124	26	7	45
Walker	Wal1997	13.4	6.7	4.8	54	97	45	46	70
Boulder	Bou1964	10.7	9.0	6.5	18	162	30	30	10
Cliff Valley	CV1964	16.0	6.9	5.8	33	177	69	26	5
West Fork Seiad	WFS1964	9.2	6.9	5.5	16	197	60	48	38
Doolittle	Doo1850	14.2	5.8	5.5	37	128	5	0	1
Upper Elk	Elk1850	12.0	5.4	5.4	32	116	4	0	0

Note: Site code is composed of a two- or three-letter abbreviation of the stream name and the year of the most recent debris flow.

this study is the need to understand how past and present forest management practices in mountainous watersheds can affect stream ecosystems and, in particular, conservation of aquatic species such as Pacific salmon (*Oncorhynchus* spp.).

Materials and methods

Study sites

This study was conducted in 10 basins in the Klamath Mountains of northern California, USA (Fig. 1). The Klamath Mountains are extremely rugged: the total relief is 2400 m, and hillslopes steeper than 65% are common. Annual precipitation ranges from <250 mm in eastern, inland valleys to >2000 mm in the high mountains, where much of it falls as snow. Extensive road building and timber harvest occurred in the Klamath Mountains in the 20th century, with the most intense activity occurring between the 1960s and 1980s.

The most recent occurrence of widespread debris flow activity in the Klamath Mountains occurred on 1 January 1997, when an unusual rain-on-snow flood (return interval of 14–28 years) resulted in widespread and extensive flooding and landslides (de la Fuente and Elder 1998). The debris flows were typically very fluid, allowing them to flow downslope into stream channels, where they mobilized bed and bank material (de la Fuente and Elder 1998). Debris flows rarely terminated in discrete deposits. Rather, in mainstem channels, debris flows often transitioned into debris floods, causing extensive reworking of channels and valley floor sediments for many kilometres (de la Fuente and Elder 1998). For simplicity, in this paper we refer to these complex debris flow – debris flood events as debris flows. It is not possible to distinguish between the two processes from air photos, which we used to map the occurrence of altered streams. Additionally, debris flows and debris floods both result in mobilization of surficial sediment deposits and vegetation removal from valley floors (Pierson and Costa 1987), which are the key disturbances being investigated in this study.

Based on initial field reconnaissance, we selected five basins that had major debris flows during the January 1997 flood (hereafter referred to as "recent debris flow streams") and five basins that did not have recent debris flows (hereafter referred to as "older debris flow streams") (Table 1). Within each basin, we selected one 200 m long (~40× bankfull width) study reach that we hypothesized was susceptible to debris flow scour (channel slope >5%; Stock and Dietrich 2003). We selected basins and reaches that were as similar as possible with regard to basin area (~10–20 km^2), channel width (~5 m), channel slope (5%–9%), and elevation (500–900 m), within the same geographic region (Table 1; Fig. 2). This slope class coincides with the domain where step–pool alluvial channel morphologies are expected to occur (channel slope, 4%–10%; Montgomery and Buffington 1997). Study streams are referred to by a site code composed of a two- or three-letter abbreviation of the stream name and the year of the most recent debris flow (e.g., Mid1997 for Middle Creek, CV1964 for Cliff Valley Creek) (Table 1).

We surveyed channel and valley morphology, riparian conditions, stream physical and biological conditions, and biological communities at the study sites between 2005 and 2007. Many of the sites were inaccessible for large portions of the year, usually from November through April. Consequently, biological sampling was conducted in August 2005, May, July, and September 2006, and June 2007. These sampling events are referred to as summer 2005, spring 2006, summer 2006, fall 2006, and summer 2007, respectively.

During the course of this study, a major flood occurred on 30–31 December 2005 (hereafter referred to as the 2005 flood). Despite the fact that rainfall totals and peak streamflows were roughly similar between the 1997 and 2005 events, very few landslides or debris flows occurred in the Klamath Mountains in 2005. The reasons for these differences in hillslope instability are not well understood but may reflect a greater contribution of snowmelt in 1997 than in 2005 or local variations in rainfall intensities.

Comparability of study sites

To assess the validity of using a space-for-time substitution (Pickett 1989) to study the ecological effects of debris flows, we examined the comparability of the 10 study basins and reaches with regard to geomorphic setting and debris flow potential. The slope of hillslopes and upstream channels is an important determinant of debris flow potential (Benda and Cundy 1990; Stock and Dietrich 2003; May 2007). Thus, we plotted longitudinal profiles of mainstem channels for each study basin (from the downstream conflu-

ence with a large river to the upper extent of the channel network) by measuring slopes between adjacent contour lines on 1 : 24 000 topographic maps with a ruler (Stock and Dietrich 2003). We also determined if there were differences in bedrock types, geomorphic features (e.g., deep-seated landslides, inner gorges, glaciated basins, glacial deposits), or hillslope gradients among the study basins, based on existing GIS layers created by geologists at the Klamath National Forest.

Debris flow chronology

We examined a sequence of aerial photographs (1944, 1955, 1964, 1965, 1974, 1987, 1997) of the study basins and conducted field surveys of study reaches to determine each basin's history of debris flows. For each air photo sequence, we determined the extent of the stream network upstream of the study reach that had evidence of recent mobilization of the bed and (or) valley floor. Determination of bed mobilization from air photos was based on (*i*) absence of riparian and valley floor vegetation or evidence of recent vegetation removal and (*ii*) fresh and usually lighter-colored substrate in stream channels. Field surveys were used to verify and expand upon inferences from the air photo analysis. We identified debris flow and debris flood deposits in the field based on the presence of unsorted, matrix-supported clasts (debris flow) or poorly sorted, clast-supported (debris flood) deposits containing large clasts of boulders and cobble. We estimated the time since the previous debris flow in the study reach by aging trees located along channel margins and on terraces. Where possible, we cored and counted growth rings from trees from even-aged stands, which were assumed to reflect the minimum age of the valley floor deposit.

Physical and chemical characteristics

We conducted geomorphic surveys of the study reaches in summer 2005 (prior to the December 2005 flood) and in summer 2007. We surveyed longitudinal profiles and cross sections and quantified the number and height of channel-spanning alluvial steps (Grant et al. 1990; Chin 1998), measured from the base of the dominant step-forming grains to the mean height of the top of the step. In all pools (>0.5 m deep), we measured the maximum depth and average length and width and described the dominant pool-forming factor (i.e., boulder, bedrock, alluvial step, or large wood). Streambed grain size was estimated with pebble counts of >200 particles.

We collected 500 mL water samples from each stream in summer 2006. Samples were filtered through glass-fiber filters (Whatman GF/C, 1.2 μm pore size) and analyzed for dissolved organic carbon (DOC), dissolved inorganic nitrogen (nitrate + nitrite), and dissolved phosphorus. We did not measure dissolved ammonium because reported concentrations in nearby small, undisturbed, forested streams were very low and a small fraction of the total inorganic nitrogen. Chemical analyses were performed by the Cooperative Chemistry Analytical Laboratory of the US Forest Service Pacific Northwest Research Station and Oregon State University (Corvallis, Oregon).

We monitored stream temperatures at 15 min intervals during the summer season (12 June – 23 September) using Hobo Water Temp Pro temperature loggers (Onset Corporation, Bourne, Massachusetts).

Riparian vegetation and organic matter

In summer 2005 and summer 2007, we surveyed riparian trees in 5 m × 5 m plots located adjacent to both sides of the bankfull channel at the beginning (0 m), middle (100 m), and end (200 m) of each study reach. We measured the diameter-at-breast-height (DBH) and identified the species of every tree greater than 1.5 m tall. We surveyed canopy cover with digital photography in summer 2005, spring 2006, and fall 2006, using methods modified from Engelbrecht and Herz (2001).

In summer 2005 and summer 2007, we surveyed the number and volume of all pieces of large wood (LW) greater than 10 cm diameter and 1.5 m long located within the bankfull channel of each study reach. We sampled coarse benthic organic matter (CBOM) concurrently with benthic macroinvertebrates (see below) from eight randomly selected 0.09 m^2 (1.0 ft^2) locations in riffle habitats in summer 2005, spring 2006, summer 2006, and fall 2006. In the laboratory, we removed benthic macroinvertebrates from the CBOM samples under a dissecting microscope. CBOM retained on a 1 mm sieve was dried at 75 °C for several hours and weighed.

Periphyton biomass and productivity

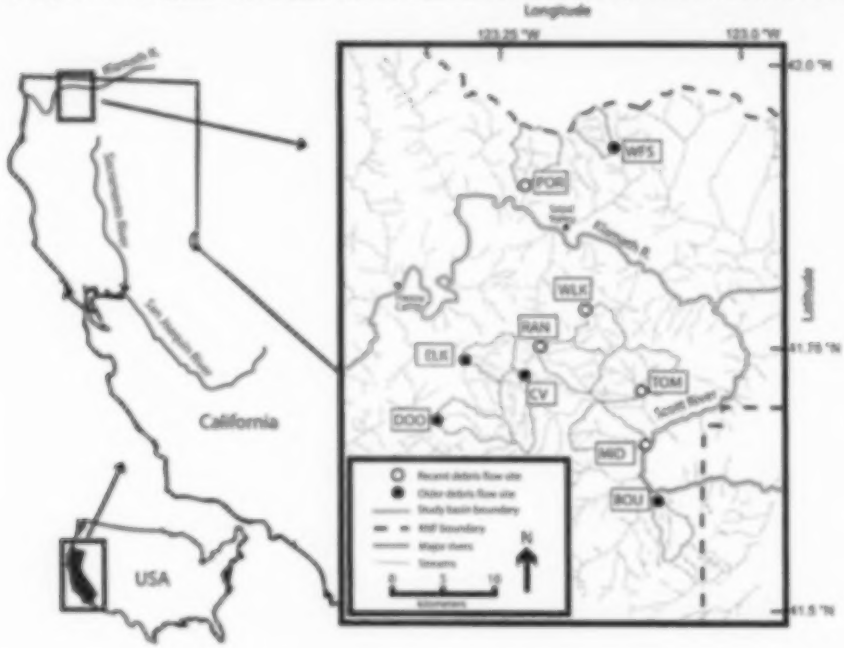
We measured ash-free dry mass (AFDM) of composited samples of periphyton collected from 10 randomly selected cobble-sized stones in summer 2005, spring 2006, summer 2006, and fall 2006. Prior to sampling, stones were gently rinsed in the stream to remove unattached, nonalgal organic particles. We vigorously scrubbed a 12 cm^2 area on the top surface of each stone with a toothbrush for 1 min. Loosened periphyton was rinsed with water and collected in a plastic pan. The resulting slurry of periphyton and water, which ranged from 250–450 mL, was vigorously shaken to homogenize the sample. We filtered a 25 mL subsample through a glass-fiber filter (Whatman GF/C, 1.2 μm pore size) using a hand-operated vacuum pump. Filters were placed in a vial wrapped in aluminum foil and kept on ice until they were dried at 60 °C for 24 h, weighed (±1.0 mg), ashed at 500 °C for 2 h, and reweighed.

Dissolved oxygen was measured at 15 min intervals for 48 h at each site in summer 2006 with a sonde equipped with dissolved oxygen and temperature–conductivity probes (model 6600, Yellow Springs International, Ohio). We had planned on determining primary production using the single-station approach (Bott 2006), but dissolved oxygen levels remained at 100% saturation throughout the night and early morning hours in every stream, making it impossible to calculate community respiration. Consequently, we plotted diel dissolved oxygen curves and used daily maxima as a general indicator of productivity.

Aquatic animals

We sampled benthic macroinvertebrates (and benthic organic matter, see above) in summer 2005, spring 2006, summer 2006, and fall 2006 from eight randomly selected 0.09 m^2 (1.0 ft^2) locations in riffle habitats using a 500 μm mesh D-frame net. Although we desired quantitative data on

Fig. 2. Location of the study sites in the Klamath Mountains, northern California, USA. Study sites are identified with two- or three-letter abbreviations (Table 1). Open circles represent recent debris flow streams and solid circles represent older debris flow streams.



the abundance of invertebrates and organic matter, the coarse-grained, rough streambeds made it impractical to use a sampling frame (i.e., Surber sampler). Instead, we simulated the benefits of a sampling frame by (*i*) delimiting the sampling area with a graduated rod and (*ii*) positioning our boots on each side of the D-frame net to direct streamflow into the net. High water velocities in our study streams also contributed to high capture efficiencies, which we visually estimated to be consistently greater than 90%. Benthic macroinvertebrates were separated from CBOM under a dissecting microscope at 10× magnification. Subsampling and identifications were performed by the National Aquatic Monitoring Center (Logan, Utah) using standard bioassessment protocols (Vinson and Hawkins 1996) and taxonomic levels (insects to genus or species). From the raw taxonomic data, we calculated standard biological metrics related to richness, abundance, diversity, tolerance, feeding group, and habit.

We performed snorkel surveys for large animals in all stream habitats with a minimum water depth of 0.3 m and a minimum area of 2.0 m^2 in summer 2006, fall 2006, and summer 2007. All snorkel surveys were performed between 1100 and 1500. We identified each large animal and visually estimated the body length to the nearest 2.5 cm. The three species that we observed were *Oncorhynchus mykiss* (steelhead or rainbow trout), *Dicamptodon tenebrosus* (coastal giant salamander), and *Pacifastacus leniusculus* (signal crayfish).

Larvae of *Ascaphus trui* (coastal tailed frog) were unintentionally collected in D-nets while sampling for benthic macroinvertebrates (see above). Upon capture, *A. trui* larvae were tallied and released.

Statistical analyses

The relationships between stream environmental and ecological variables and debris flows were analyzed in two ways: (*i*) regressions of variables versus years since debris flow, and (*ii*) comparisons of variables between streams that experienced debris flows in 1997 (recent debris flow streams) and streams that experienced debris flows in the more distant past (older debris flow streams). We tested the significance of differences between recent and older debris flow streams with *t* tests.

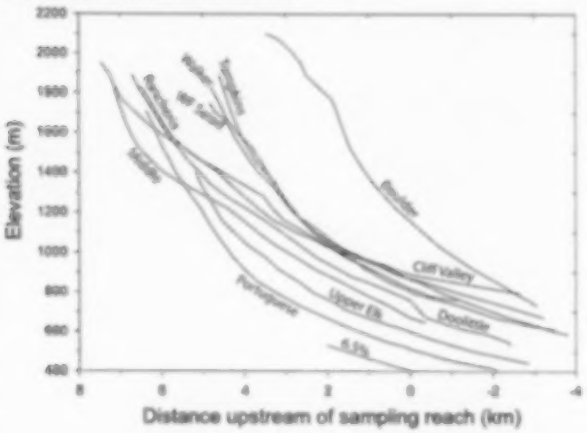
Results

Comparability of study sites

Longitudinal profiles of mainstem stream channels in the study basins are similarly steep (>6.5%), suggesting that similar geomorphic processes are likely to occur in the study basins (Fig. 3). Mainstem channel slopes downstream of the study reaches are typically 5%–7%, suggesting that debris flow deposition is possible in these reaches (Benda and Cundy 1990; Stock and Dietrich 2003). The dominant bedrock lithologies in the study basins include granitic and ultramafic rocks; granitic rocks are mostly confined to basins in the eastern portion of the study area (Mid1997, Tom1997, Wal1997, and Ran1997; for site codes, see Table 1). Large portions of most basins are underlain by potentially unstable slopes such as dormant, deep-seated landslides, inner gorges, and steep slopes, and significant portions of headwater hillslopes in three basins have been glaciated (Cover 2008).

Although there are some differences in lithology and geomorphic features between the basins, we believe that they are comparable with respect to the potential for channel-

Fig. 3. Longitudinal profiles of the mainstem channel in each study basin. Profile distances are standardized with respect to the downstream end of the study reaches. For reference, a profile with a slope of 6.5% (the critical slope of debris flow deposition of Benda and Cundy (1990)) is also shown.



scouring debris flows and debris floods. The ubiquitous steep (>65%), soil-mantled headwater hillslopes and high-gradient mainstem channels (>6.5%) present in all of the study basins suggests that, as in much of the humid Pacific Northwest, shallow landslides can trigger debris flows that travel down steep stream channels as debris floods. This assumption was confirmed during the course of the field studies, as we found evidence for recent or historic (<200 years) debris flow and debris flood deposits in every study reach.

Debris flow chronology

Air photo analysis revealed that large portions of the stream networks in each of the five basins identified as recent debris flow streams were affected by debris flows or debris floods in 1997 (Table 1). Field evidence confirmed that surficial sediment deposits in mainstem valley floors were nearly completely mobilized and stripped of vegetation (Fig. 4*a*). Based on the air photo record, most of the basins that had debris flows in 1997 also had events in 1964 (Table 1). However, there was little evidence that extensive debris flows had occurred in these five basins prior to the 1964 flood event. Dense stands of large conifer and hardwood trees are visible in the mainstem valley floors of most basins in the 1944 air photos, similar to the current valley floor vegetation in basins where debris flows have not occurred for >150 years (Doo1850 and Elk1850). Thus, the debris flow events of 1964 and 1997 most likely followed a period of relative quiescence in these basins that lasted at least 100 years.

In contrast with the recent debris flow streams, there was little evidence for recent (i.e., 1997) channel scour in the mainstem channels of the five basins that were classified as older debris flow streams. Three of the older debris flow streams (Bou1964, CV1964, and WFS1964) were extensively scoured by debris flows in 1964. Although these streams were partially remobilized by the 1974 flood (Table 1), we classified the streams as last having debris flows in 1964 because there was much more sediment delivery and much wider valley-floor disturbances in 1964 than in 1974. Based on the presence of 30- to 40-year-old alder trees along the stream margins, both streams appear to have been quite stable since the 1974 flood (Fig. 4*c*), although the headwaters of one basin (WFS1964) experienced some localized scour as a result of the 1997 flood (Table 1). There was no evidence in the air photo record of debris flow activity in two basins (Doo1850 and Elk1850), and field evidence indicates the presence of very old debris flow deposits. Large conifers near the edges of the bankfull channels were dated to 150 to 200 years old, indicating that the channel has not been disturbed by debris flows or floods for several centuries (Fig. 4*d*). Based on this evidence, we selected 1850 as most recent possible date for a debris flow event for both streams.

Stream physical and chemical characteristics

Recent debris flow streams had significantly fewer alluvial steps (6.5 ± 1.8 steps per reach, mean ± standard error (SE)) than older debris flow streams (14.0 ± 2.9) in August 2005 (t test, $p = 0.05$). As a result of the 2005 flood, however, many alluvial steps were created in several recent debris flow streams. Most notably, the number of steps in Wal1997 increased from five prior to the 2005 flood to 19 following the flood, including two 1 m tall alluvial steps formed around boulders. As a consequence of step formation associated with the 2005 flood, in 2007 there was no difference in alluvial step frequency between recent debris flow streams (11.3 ± 3.8) and older debris flow streams (14.8 ± 1.7) (t test, $p = 0.43$).

The number and total area of pools per reach was similar in recent debris flow streams and older debris flow streams, but pool-forming factors varied considerably. In older debris flow streams, most pools were formed by alluvial steps (2.9 ± 1.1 pools per reach) and large wood (1.3 ± 0.4), whereas boulders (2.9 ± 0.6) and bedrock (2.4 ± 1.0) were the most common pool-forming factors in recent debris flow streams.

Bed surface grain size varied among the 10 streams irrespective of debris flow history. The steepest streams (Bou1964, Mid1997) had the coarsest beds (D50 > 180 mm; Table 1) and the most exposed bedrock (25% and 21%, respectively). The finest-grained streambeds (D50 < 100 mm) occurred in recent debris flow streams with wide alluvial valleys (Wal1997, Ran1997). We did not observe any quantitative or qualitative differences in levels of fine sediment between the recent debris flow streams and older debris flow streams.

There was no difference in dissolved inorganic nitrogen (nitrate + nitrite) and dissolved phosphorus concentrations between recent debris flow streams and older debris flow streams. Inorganic nitrogen was present at lower concentrations (range, 0.001–0.012 mg·L^{-1}) than total phosphorus (range, 0.011–0.023 mg·L^{-1}) in all samples. Very low concentrations of inorganic nitrogen were measured in Doo1850 (0.001 mg·L^{-1}), Wal1997 (0.001 mg·L^{-1}), and Ran1997 (0.002 mg·L^{-1}), while the highest concentrations were measured in Mid1997 and CV1964 (0.012 mg·L^{-1}). Dissolved organic carbon (DOC) concentrations were significantly higher in recent debris flow streams (0.74 ±

Fig. 4. Photographs of representative study reaches: (*a*) Portuguese Creek, nine years after debris flow; (*b*) Walker Creek, nine years after debris flow; (*c*) Cliff Valley Creek, 42 years after debris flow; (*d*) Doolittle Creek, ~150 years after debris flow.

0.05 mg·L^{-1}, mean ± SE) than in older debris flow streams (0.54 ± 0.03 mg·L^{-1}) (*t* test, $p = 0.008$).

Maximum temperatures and average diel temperature ranges were higher in recent debris flow streams than in older debris flow streams in summer 2006. Maximum temperatures were >19 °C (range, 19.1–23.9 °C) in recent debris flow streams, whereas maximum temperatures in older debris flow streams were always <19 °C (range, 14.7–18.6 °C) (*t* test, $p = 0.01$) (Fig. 5*a*). Average summer diel temperature ranges were significantly greater at recent debris flow streams (3.23 ± 0.42, mean ± SE) than at older debris flow streams (2.0 ± 0.06) (*t* test, $p = 0.01$). Maximum temperature and average diel temperature range were both inversely correlated with canopy cover.

Riparian vegetation and organic matter

Species richness of riparian trees was lowest at recent debris flow streams and highest at the oldest debris flow streams (Fig. 5*b*). Riparian assemblages in recent debris flow streams were dominated by even-aged stands of *Alnus rhombifolia* (white alder), which made up >90% of stems at four of the five recent debris flow streams. Other species present in recent debris flow streams were *Acer macrophyllum* (bigleaf maple) and *Salix* spp. (willow). The three streams that had debris flows in 1964 were dominated by mid-sized (20–40 cm DBH) alder trees, although some small conifers (DBH < 15 cm), including incense cedar (*Calocedrus decurrens*) and Douglas-fir (*Pseudotsuga menziesii*), were also present. In addition to the species mentioned above, riparian zones in the oldest debris flow streams contained trees such as *Arbutus menziesii* (Pacific madrone), *Taxus brevifolia* (Pacific yew), and *Lithocarpus densiflorus* (tanoak).

Tree basal area was also lowest in recent debris flow streams and highest in the oldest debris flow streams (Fig. 5*c*). Densities of alder were quite high (2000–8000 ha^{-1}) at recent debris flow streams, but tree basal areas were quite low because all trees in the riparian zone were very young and had small diameters (<15 cm DBH). The main contributors to the large basal areas at the oldest debris flow streams were a few very large (>100 cm DBH) Douglas-fir trees.

In 2005, there was no difference in canopy cover between the recent debris flow streams and older debris flow streams (*t* test, $p = 0.66$), but following the 2005 flood, canopy cover was significantly lower at the recent debris flow streams (*t* test, $p = 0.002$) (Fig. 5*d*). Prior to the flood, canopy cover was highest at Wal1997 (80%) and Tom1997 (75%), where dense stands of alder were angled towards the center of the stream (Fig. 4*b*). Other recent debris flow streams (Mid1997 and Ran1997) had moderately low canopy cover (58% and 59%, respectively) because vegetation was absent on exposed bedrock, usually around pools. Older debris flow streams had less variation in canopy cover (62%–72%). As a result of the 2005 flood, canopy cover at three recent de-

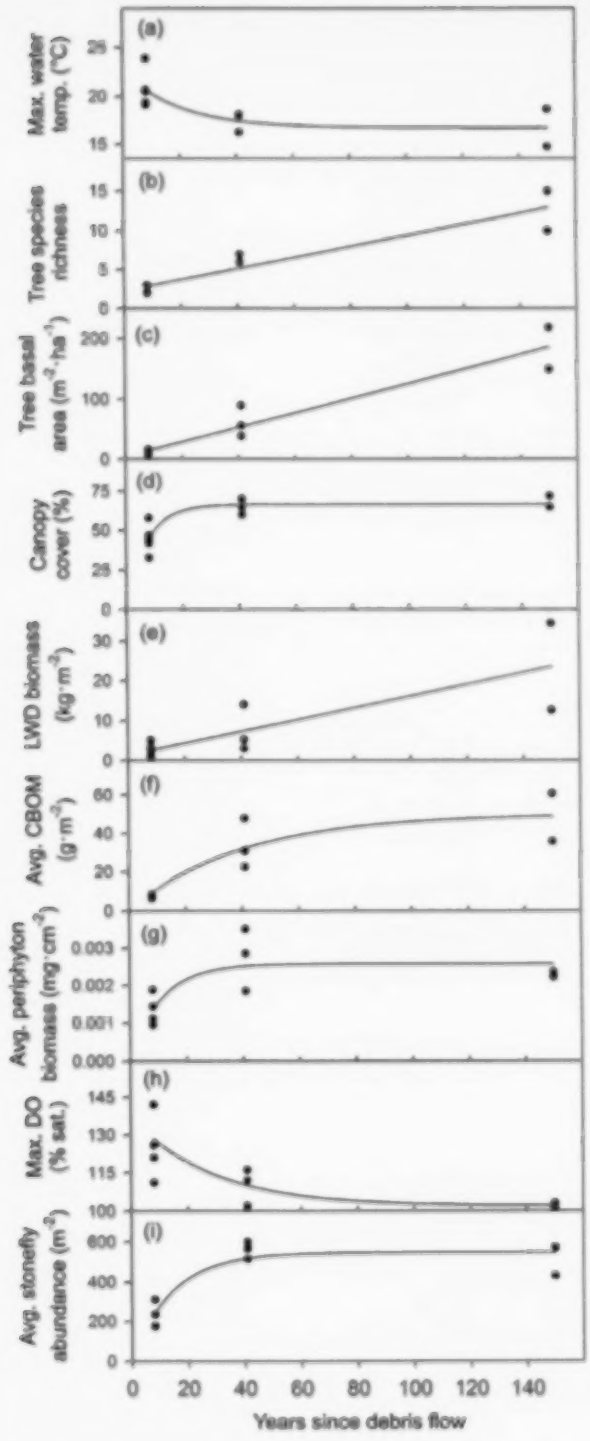


Fig. 5. Variation in ecological parameters with time since the most recent debris flow: (*a*) maximum summer water temperature in 2006; (*b*) species richness of riparian tree assemblages in 2005; (*c*) basal area of riparian trees in 2005; (*d*) average riparian canopy cover; (*e*) biomass of large wood in 2005; (*f*) average biomass of coarse benthic organic matter (CBOM); (*g*) average ash-free dry mass of periphyton, (*h*) maximum dissolved oxygen (DO) saturation in mid-summer, 2006; (*i*) average abundance of stoneflies in benthic samples. Graphs were fit with the following types of equations: (*a* and *h*) exponential decay; (*b*, *c*, and *e*) linear; and (*d*, *f*, *g*, and *i*) exponential rise to maxima.

bris flow streams (Wal1997, Ran1997, and Tom1997) decreased substantially (to 33%, 42%, and 47%, respectively) because of the loss of young alder trees (>40% mortality) in the riparian zone.

Large wood biomass increased with time since debris flow (Fig. 5*e*). The oldest debris flow streams, Doo1850 and Elk1850, had large amounts of LW (14–37 kg·m^{-2}). There was little change in LW levels in Doo1850 as a result of the 2005 flood, but a significant portion of LW (36%) present in Elk1850 in 2005 was transported out of the reach. LW biomass at recent debris flow streams and streams affected by 1964 debris flows was uniformly low (<7 kg·m^{-2}). Most of the wood in recent debris flow streams was small (<5 cm DBH), young (post-1997) alders that were recruited during the 2005 flood.

There was significantly more CBOM in the older debris flow streams (range, 22.6–60.6 g·m^{-2}) than in the recent debris flow streams (range, 6.5–8.5 g·m^{-2}) averaged over all seasons (*t* test, $p = 0.001$) (Fig. 5*f*). The highest levels of benthic organic matter (>40 g·m^{-2}) were consistently found in streams that had not had debris flows for >150 years. There was relatively little variation in CBOM between 2005 and 2006 or between seasons within the debris flow classes.

Periphyton and primary productivity

Periphyton ash-free dry mass (AFDM) was generally higher in older debris flow streams than in recent debris flow streams (Fig. 5*g*), although this pattern varied seasonally. AFDM was significantly greater in older debris flow streams in summer 2005 and fall 2006, but in summer 2006, there were similarly low levels in both groups of streams. AFDM was consistently low (<1.5 μg·cm^{-2}) at most recent debris flow streams.

Recent debris flow streams exhibited large, short-lived peaks in dissolved oxygen during the mid-day hours (1100–1400) (Fig. 5*h*). For example, dissolved oxygen levels were greater than 130% of saturation for 1–2 h at Mid1997 and Tom1997. The 1964 debris flow streams had moderate dissolved oxygen curves (peaks >110% of saturation), with the exception of CV1964, which had very little oxygen production. The oldest debris flow streams, Elk1850 and Doo1850, had very subdued dissolved oxygen curves (peaks <103% of saturation). There was a negative relationship between canopy cover and maximum dissolved oxygen, although one site (Ran1997) had low canopy cover and low oxygen maxima, suggesting that a factor other than sunlight may have limited primary productivity.

Aquatic animals

Benthic macroinvertebrate assemblages were substantially different between recent debris flow streams and older debris flow streams throughout the sampling period, although

Table 2. *p* values of statistically significant differences in benthic macroinvertebrate metrics between recent debris flow streams and older debris flow streams by season.

	p values			
Metric	Summer 2005	Spring 2006	Summer 2006	Fall 2006
Taxa richness		0.01		0.04
Ephemeroptera taxa				0.015
Ephemeroptera abundance	0.04			
Plecoptera taxa		0.01		0.007
Plecoptera abundance	0.006	0.04		0.001
Megaloptera taxa		0.05		
Megaloptera abundance		0.01		
Diptera taxa		0.05		
Diptera abundance				0.003
Shredder taxa	0.05			0.003
Scraper abundance				0.04
Predator taxa		0.01		
Clinger taxa		0.03		
Long-lived taxa		0.01		

Note: For all statistically significant differences, values were higher at older debris flow streams than at recent debris flow streams.

Table 3. Taxa that were significantly more abundant at recent debris flow streams or older debris flow streams, and *p* values < 0.05 by season and overall.

	Average abundance (m^{-2} (SE))		*p* values < 0.05				
Taxa	Recent debris flow streams	Older debris flow streams	Summer 2005	Spring 2006	Summer 2006	Fall 2006	Overall
Ampumixis dispar (C)	48.9 (8.9)	25.0 (6.0)			0.04		0.03
Optioservus (C)	19.8 (6.1)	3.7 (1.7)		0.04			0.02
Glossosomatidae (T)	110.2 (46.5)	22.6 (6.7)	0.04				
Heptageniidae (E)	325.0 (84.2)	569.1 (113.0)		0.03			
Orohermes (M)	0.9 (0.5)	6.9 (2.5)		0.03	0.008		0.03
Chloroperlidae (P)	8.2 (2.2)	47.5 (9.3)		0.05			0.0002
Doroneuria baumanni (P)	1.9 (0.8)	16.7 (4.5)		0.01		0.04	0.002
Pteronarcys (P)	0.3 (0.2)	6.3 (1.8)		0.03	0.05		0.002
Visoka cataractae (P)	0.1 (0.1)	7.4 (1.8)	0.05			0.01	0.0002
Yoraperla (P)	3.4 (1.4)	80.3 (17.7)	0.006			0.008	0.0001
Zapada columbiana (P)	47.0 (22.3)	155.2 (46.7)	0.05				0.04
Wormaldia (T)	0.7 (0.4)	20.1 (8.2)					0.02

Note: Abundances are averaged over streams and seasons. Letters in parentheses after taxa refer to insect orders: C, Coleoptera; E, Ephemeroptera; M, Megaloptera; P, Plecoptera; T, Trichoptera. SE, standard error.

differences were especially apparent following the 2005 flood (Tables 2, 3). Five months after the 2005 flood, taxonomic richness was significantly lower at recent debris flow streams (48 ± 4) than at older debris flow streams (63 ± 2) (*t* test, $p = 0.01$) (Table 2). Total taxa richness was especially low (37) at Wal1997, where flood impacts were the most severe. We visited Wal1997 in early February, a little over a month after the 2005 flood, and found that benthic macroinvertebrate abundance (~50 m^{-2}) and taxonomic richness (4) were both extremely low.

Stoneflies and other shredding invertebrates were significantly more abundant at older debris flow streams than at recent debris flow streams (Table 3; Fig. 5*i*). Prior to the 2005 flood, shredder abundance was much lower (<70 m^{-2}) in four out of five recent debris flow streams than in older debris flow streams (448 ± 71 m^{-2}, mean ± SE), whereas stoneflies were three times more abundant in older debris flow streams (698 ± 105 m^{-2}) than in recent debris flow streams (230 ± 65 m^{-2}) (*t* test, $p = 0.006$). Overall, chloroperlid stoneflies were approximately six times more abundant in older debris flow streams than in recent debris flow streams, and stoneflies such as *Visoka cataractae* (Nemouridae), *Yoraperla* (Peltoperlidae), *Doroneuria baumanni* (Perlidae), and *Pteronarcys* (Pteronarcyidae) were abundant at older debris flow streams but absent or rare at recent debris flow streams (Table 3).

The composition and density of grazing invertebrates varied with disturbance history, canopy cover, and year. In 2005, glossosomatid caddisflies (*Glossosoma* and *Agapetus*) were more abundant (>450 m^{-2}) than heptageniid mayflies (*Cinygmula*, *Epeorus*, *Ironodes*, and *Rhithrogena*) (<100 m^{-2}) at recent debris flow sites with relatively open canopies (canopy cover < 60%; Ran1997, Por1997, and Mid1997). Where canopy cover was moderate to high

(>62%), heptageniid mayflies (350 m^{-2}, mean) were more abundant than glossosomatid caddisflies (<100 m^{-2}). Following the 2005 flood, glossosomatid densities were uniformly low (<55 m^{-2}) at every site in all seasons, whereas heptageniid densities were high (>250 m^{-2}) at eight out of 10 sites in all seasons. In summer 2007, following a winter season that lacked large floods (return interval of the largest flood, 1.3 years), glossosomatid densities (estimated visually) were again quite high at recent debris flow streams and low at older debris flow streams.

Oncorhynchus mykiss were observed in every reach during every sampling event, but abundances varied considerably between streams. We observed the highest numbers (>60) of *O. mykiss* in 2006 in Ran1997 and Mid1997, which both experienced debris flows in 1997, and in Elk1850, which has not had debris flows for >150 years. Although fish abundance in most streams ranged from 10 to 40 per survey, only two to four fish were ever observed in Wal1997. With the exception of Wal1997, we consistently observed the fewest fish (<20) in streams that had debris flows in 1964 but not in 1997 (WFS1964, Bou1964, CV1964).

Ascaphus frogs and *Pacifastacus* crayfish were common in older debris flow streams (average individuals per site visit, 1.1 and 3.1, respectively), but we never observed either species in recent debris flow streams. Similarly, *Dicamptodon* salamanders were fairly common at older debris flow sites (average individuals per site visit, 1.5), but only one individual was observed at any of the recent debris flow sites (average individuals per site visit, 0.07). Within the older debris flow streams, *Ascaphus* was more abundant in streams that had debris flows in 1964, whereas *Pacifastacus* was more abundant in streams that have not had debris flows in the past century.

Discussion

Debris flows and regional topography

Debris flows and debris floods can affect large portions of stream networks in mid-sized (10–20 km^2) drainage basins in the Klamath Mountains. As a result of the 1997 floods, we observed extensive mobilization of sediments on the surfaces of valley floors and removal of riparian forests along streams for linear distances >5 km, often extending from near drainage divides down to tributary mouths at major rivers. In contrast, most previous studies have described debris flow disturbances in first- or second-order channels in small basins (<1 km^2) (e.g., Benda and Cundy 1990; Kiffney et al. 2004) or along short lengths (100–1000 m) of mainstem channels in larger basins affected by debris flow deposition (e.g., Lamberti et al. 1991; Snyder and Johnson 2006). Although the long debris flow run-out distances that we observed could reflect more intense flood events produced by rain-on-snow hydrology, it seems likely that the high steepness and low concavity of stream networks in the Klamath Mountains is a critical factor (May 2007). The primary control on debris flow run-out distance is channel slope (Benda and Cundy 1990; Stock and Dietrich 2003), although other factors such as tributary junction angle (Benda and Cundy 1990), large wood (Lancaster et al. 2003), and the presence of large riparian trees (Johnson et al. 2000) can also be important. Stock and Dietrich (2003) found that debris flows were often deposited in channels with slopes between 3% and 10% in mountainous basins around the world, whereas Benda and Cundy (1990) found a critical slope of 6.5% for debris flow deposition in the Oregon Coast Range.

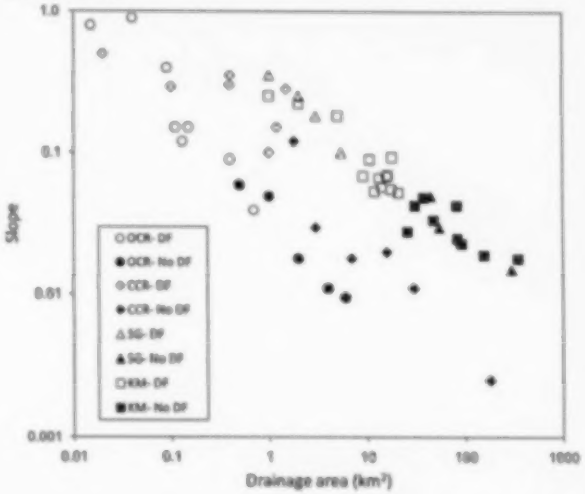


Fig. 6. Slope and drainage area of stream channels of the Pacific coast of the United States. Channels that experienced debris flows are shown with open symbols; channels that did not experience debris flows are shown with solid symbols. Note the order of magnitude variation in slope among streams with similar drainage areas. All data are from Stock and Dietrich (2003) unless otherwise indicated. OCR, Oregon Coast Range; CCR, California Coast Range; SG, San Gabriel Mountains of southern California; KM, Klamath Mountains of northern California (data from Cover (2008) and this study).

Relationships between channel slope and drainage area vary considerably among mountainous regions, even within the Pacific Coastal ecoregion of the United States. For example, as basin size increases above 0.1 km^2 in the Oregon Coast Range, channel slope decreases rapidly (Fig. 6). Consequently, debris flows often scour steep (30%–40%) headwater channels down to bedrock for short distances (200–1000 m) and are quickly deposited at tributary junctions of mainstem rivers (Benda and Cundy 1990; May and Gresswell 2004). In contrast, channels in the Klamath Mountains of northern California and the San Gabriel Mountains of southern California are much steeper for a given drainage area (Fig. 6). In the Klamath Mountains, basins as large as 35 km^2 have mainstem channel slopes >6.5%, whereas basins as large as 100 km^2 have mainstem channel slopes >3.5% (Fig. 6). In these areas, debris flows are more likely to transform into debris floods, with total run-out lengths of 1–10 km (Morton and Campbell 1974; de la Fuente and Elder 1998). Unlike small headwater channels of the Oregon Coast Range that are often scoured to bedrock, the larger channels of the Klamath Mountains are usually transport zones during debris flows and debris floods, resulting in mobilization and deposition of alluvial sediment.

Regional differences in topography result in considerable variability in the spatial extent of debris flow process zones

and the geomorphic effects of debris flows, which can have tremendous consequences on stream ecosystem structure, food web dynamics, and the conservation of aquatic species. For example, channels that are scoured down to bedrock will have a very different recovery trajectory than transport reaches, because the re-establishment of a riparian forest requires sufficient alluvial substrates to support the colonization and growth of pioneer tree species (May 2002). Portions of a channel network that are susceptible to debris flow scour, transport, and deposition can be predicted for mountain drainage basins using information that is easily collected from topographic maps or digital elevation models such as slope, drainage area, and concavity (Stock and Dietrich 2003; Benda et al. 2005; May 2007).

Channel morphology and stability

We observed substantial differences in channel morphology between recent debris flow streams and older debris flow streams, reflecting a lack of both large wood and channel bed organization by fluvial processes. Although stream channels affected by debris flows in 1997 had fewer alluvial steps, a flood in 2005 (return interval, 10–15 years) resulted in the formation of many additional alluvial steps. Others have reported that step formation requires flood flows with return intervals of 5–100 years or more, with most step formation occurring at flows with return intervals >30 years (Grant et al. 1990; Chin 1998).

It may take a century or more for substantial amounts of large wood (LW) to accumulate in our study streams following debris flows. Large wood biomass in the oldest debris flow streams (14–37 kg·m^{-2}) was comparable with levels in other small streams draining undisturbed, mature conifer forests of the Pacific Northwest (31–74 kg·m^{-2}; Harmon et al. 1986). Wood biomass in streams affected by debris flows in 1997 and (or) 1964, however, was much lower (<7 kg·m^{-2}). Following disturbance events that remove LW and riparian vegetation, recruitment of stable LW is likely to be impaired for at least a century until conifer trees become established and mature. In smaller, steeper streams of the Oregon Coast Range, LW continued to accumulate for over 150 years following debris flow scour (May and Gresswell 2003). Given the tremendous importance of LW to the structure and function of stream ecosystems in the Pacific Northwest (e.g., Harmon et al. 1986), removal of LW may be one of the most significant and long-lasting effects of debris flows and debris floods. Wood is often a more important pool-forming factor in old-growth streams than in streams draining harvested forests (Montgomery et al. 2003). We found that as a result of the lack of large wood and alluvial steps in recent debris flow streams, pools in these streams were more often formed by boulders and bedrock than by wood and alluvial steps. In streams that had not recently been affected by debris flows, wood and alluvial steps were the dominant pool-forming factors.

Our observations suggest that stream channels and riparian forests are more susceptible to disturbance by floods in the years following debris flows. For example, the 2005 flood resulted in the removal of large numbers of young alder trees, extensive bank erosion, and channel avulsion in alluvial streams that had debris flows in 1997, whereas older debris flow streams exhibited no changes in channel morphology. One possible reason for this difference is the effect of large wood on channel roughness. Large wood can greatly reduce flow velocity and sediment transport during floods, as well as the run-out length of debris flows (Harmon et al. 1986; Johnson et al. 2000; Lancaster et al. 2003). The lack of large wood in channels and riparian zones following debris flows may increase the susceptibility of these streams to future flood and debris flow disturbances.

Trophic dynamics and food web structure

Our observations of stored organic matter, riparian vegetation, and invertebrate consumers suggest that detritus-based food webs may take decades to recover following debris flows, especially if subsequent floods reduce riparian forests. Recent debris flow streams had three to five times less CBOM than older debris flow streams, even after nearly a decade of terrestrial inputs. We hypothesize that the timing, quality, and quantity of leaf inputs in the years following debris flows may result in reduced food resources for benthic detritivores. Alder trees can supply substantial allochthonous inputs during leaf fall in autumn, but large portions of total inputs can be exported from the system during high flow events (Richardson 1992). Conifer needles, in contrast, decompose more slowly and provide a year-round, though less nutritious, source of food for invertebrate detritivores (Richardson 1992; Richardson et al. 2004).

Many invertebrate shredders, including winter stoneflies, are food limited and require substantial food resources throughout the year (Richardson 1991; Richardson et al. 2004). In this study, we found invertebrate shredders that live for a full year or more such as the stoneflies *Zapada*, *Yoraperla*, *Sweltsa*, and *Pteronarcys* were abundant at older debris flow streams but virtually absent at recent debris flow streams. Recent debris flow streams with low levels of benthic organic matter may not be able to support detritivorous invertebrate taxa that require sustained, diverse food resources. Alternatively, differences in abundance of detritivorous stoneflies could be a result of slow recolonization rates. In any event, the lower biomass of CBOM and lower abundance and diversity of shredding invertebrates in recent debris flow streams suggests that the allochthonous portion of stream food webs remains less productive and less complex 10 years after debris flows.

Autochthonous productivity in recent debris flow streams may have been quite high, as evidenced by dissolved oxygen maxima and abundances of grazing invertebrates. Following the reductions in canopy cover at recent debris flow streams as a result of the 2005 flood, we observed much greater algal productivity, as inferred from high levels of dissolved oxygen during mid-day hours. As in other forested mountain streams of the Pacific Northwest, primary production in our study streams was likely light limited (Hill and Knight 1988; Wilzbach et al. 2005). Algal biomass (AFDM) in the study streams was usually an order of magnitude lower than levels reported in the literature for streams of the Pacific coastal ecoregion, however (e.g., Feminella and Hawkins 1995). Low levels of periphyton biomass could reflect high grazing intensity (Lamberti et al. 1989; McNeely et al. 2007). In forested Oregon streams, over 90% of algal productivity was harvested by grazers at low

light conditions (Lamberti et al. 1989). Alternatively, supersaturated dissolved oxygen concentrations may not reflect high productivity, and the high densities of invertebrates classified as grazers could suggest that "grazers" utilized alternate food sources in place of algae (McNeely et al. 2007).

Although primary productivity in most streams was light-limited, low concentrations of inorganic nitrogen (<0.002 mg·L^{-1}) could have limited or co-limited productivity at several sites (Hill and Knight 1988). Large stands of riparian alder trees can produce elevated concentrations of dissolved nitrate in stream water (Compton et al. 2003). However, some recent debris flow streams with extensive alder stands (Wal1997 and Ran1997) had very low nitrogen concentrations, suggesting that increased solar radiation at these two sites could result in rapid biological uptake of available inorganic nitrogen. In the years immediately following debris flows, we hypothesize that pioneer alders grow rapidly but canopy cover remains low, implying that increased inputs of solar radiation and nitrate could provide optimal conditions for periphyton growth.

Densities and composition of grazing invertebrates varied with canopy cover and disturbance history. In summer 2005, recent debris flow streams with open canopies contained high abundances of glossosomatid caddisflies, whereas heptageniid mayflies were the dominant grazers in older debris flow streams. Following a major flood in December 2005, caddisflies were present in low numbers throughout the year at all sites. In summer 2007, following a moderate winter, glossosomatids were again abundant in streams with open canopies. Similar patterns were observed in nearby northern California streams by Wootton et al. (1996), who found much higher densities of armored grazers (i.e., cased caddisflies) and lower densities of predator-susceptible grazers (i.e., mayflies) in regulated streams that do not experience large floods relative to unregulated systems. In the nearby Eel River of northern California, McNeely et al. (2007) found that glossosomatid caddisflies maintained densities that suppressed the algal standing stock and outcompeted heptageniid mayflies in streams with open canopies and high primary productivity. Thus, the relative abundance of heptageniids and glossosomatids depends on both radiation levels (glossosomatids are superior competitors at the higher productivity levels found in debris flow streams) and disturbance history (glossosomatid populations are reduced following severe winter floods).

Other studies have shown that increased primary productivity can be rapidly utilized by higher trophic levels. Wilzbach et al. (2005), working in small streams just west of our study area, found that removing riparian alders along 100 m long reaches of streams resulted in increased densities, biomass, and growth rates of rainbow trout (*O. mykiss*) and cutthroat trout (*O. clarki*). Likewise, Bilby and Bisson (1992) observed greater salmonid production in a clear-cut stream relative to a forested stream. In this study, there were no clear relationships between the abundance of *O. mykiss* and canopy cover or debris flow history. The highest abundances of *O. mykiss* were generally in recent debris flow streams, but high densities were also present in the oldest debris flow streams and one recent debris flow stream had the fewest fish. There are several possible explanations as to why increased primary productivity did not result in substantially greater predator abundances in recent debris flow streams. First, increased autochthonous productivity could have been offset by the decreased allochthonous productivity of recent debris flow streams. Because we did not measure leaf input or decomposition rates, it is difficult to assess the relative magnitude of the autochthonous versus allochthonous energy pathways. Second, the autochthonous energy pathway could have hit a "dead end" prior to becoming available to fish. Because their stone cases offer protection from predation, cased caddisflies can limit the transfer of autochthonous carbon to higher trophic levels, effectively sequestering algal productivity (Power 1992). Fish densities were lower in recent debris flow streams in 2007 than in 2006, possibly because glossosomatids were more abundant and consumed more of the energy base of the stream ecosystem in 2007, reducing energy transfer to higher trophic levels. However, *O. mykiss* numbers were also lower in 2007 in the oldest debris flow streams, where glossosomatids are not abundant. Lastly, increased productivity could have been offset by higher stream temperatures, which reduce salmonid growth rates.

Debris flows and aquatic biodiversity in a watershed context

Debris flow disturbances likely resulted in the extirpation of many animal species from affected drainage basins. Unlike trout, which can quickly recolonize streams after debris flows occur (Lamberti et al. 1991; Roghair et al. 2002), recolonization of some animal species may require decades or more. We did not observe tailed frogs (*Ascaphus truei*) or crayfish (*Pacifastacus*) in streams that experienced debris flows in 1997, although these species were common in older debris flow streams. The hellgrammite *Orohermes crepusculus*, which has a long-lived (4–5 years) aquatic larval stage and a short-lived (~1 week) riparian adult stage, was seven times more abundant in older debris flow streams than in recent debris flow streams. Likewise, coastal giant salamanders (*Dicamptodon tenebrosus*) were virtually absent in recent debris flow streams. Swanson et al. (1998) hypothesized that *Dicamptodon* would recolonize stream reaches affected by debris flows in 5 years. If populations were extirpated from basins, however, recolonization could take decades to centuries because source populations would have to travel over drainage divides or colonize from far downstream. Depending on their life history attributes and distribution in the drainage network, certain animals may be extremely susceptible to local extirpation by debris flows that affect large portions of the drainage network. Although not examined in this study, catastrophic disturbances that eliminate riparian vegetation and soils could also result in the extirpation of riparian wildlife such as birds, mammals, amphibians, and terrestrial invertebrates.

One of the most important impacts of debris flows on sensitive aquatic species may actually occur far downstream of debris flow transport reaches. In the mainstem Klamath River, summer maximum temperatures often exceed 25 °C, the lethal limit for most salmonids (Bartholow 2005). Consequently, juvenile salmonids rearing in the mainstem Klamath require cold-water refugia at the mouths of tributaries to survive during the summer season (Sutton et al. 2007). In this study, we documented higher maximum stream temper-

atures and greater diel temperature variation 9 years following debris flows. Johnson and Jones (2000) described increases in maximum stream temperatures of up to 7 °C in a small stream in the Oregon Cascades in the years immediately following a debris flow. We hypothesize that increased stream temperatures in tributaries will result in reduced availability and quality of cold-water refugia in the Klamath River.

Implications for forest and watershed management

We saw little evidence that our study basins had extensive debris flows in mainstem channels in the 100 years preceding 1964, but major channel-altering events occurred approximately once per decade since 1964. The increased frequency of debris flows that we observed in the second half of the 20th century corresponds with the most intense period of logging and road-building (1950s–1980s) that occurred in our study area. Timber harvest activities can increase frequencies of landslides and debris flows in the Klamath Mountains (de la Fuente and Haessig 1994) and elsewhere in the Pacific Northwest (Montgomery et al. 2000). Forest management practices may also have contributed to long debris flow run-out paths by reducing wood volumes, which promote debris jam formation and debris flow deposition; managed channels often lack large wood, and landslides and debris flows that originate from roads or harvested hillslopes often lack large trees (May 2002; Lancaster et al. 2003). However, the second half of the 20th century also coincides with a wet period with many unusually large floods. In the Salmon River, a nearby gaged stream, there were no floods with peak streamflows >1400 $m^3 \cdot s^{-1}$ between 1927 and 1954 (28 years), whereas between 1955 and 2005 (51 years), there were seven floods of this magnitude. Thus, both land use and climate likely influenced spatial and temporal patterns of debris flows in the second half of the 20th century in the Klamath Mountains.

Increased frequencies of catastrophic debris flows and debris floods could have significant impacts on the conservation of aquatic ecosystems and species in the Klamath Mountains and other forested, mountainous landscapes. In this study, we described how debris flows and debris floods play a fundamental role in structuring stream ecosystems of the Klamath Mountains. Important long-term effects of debris flows include altered channel morphology, reduced input and storage of large wood, altered organic matter cycling, loss of biodiversity, and increased stream temperatures. Each of these factors could have important direct or indirect effects on salmonid populations and other species of concern. Although catastrophic disturbances are a natural and necessary process in ecosystems, increased frequency or extent of disturbances may result in significant reductions in the availability of refugia for affected biota. For example, multiple contiguous tributary basins in the Klamath Mountains were affected by debris flows in 1997, eliminating major sources of cold-water refugia from nearly a 10 km section of the Klamath River (Cover 2008). The high proportion of tributary basins in the Klamath Mountains that have had recent debris flow disturbances suggests that undisturbed basins may play a critically important role in preserving landscape-scale biodiversity. It may be wise to prioritize restoration and conservation activities such as the removal or reconstruction of road crossings in areas that reduce the potential for catastrophic debris flows in undisturbed basins. Although current restoration plans for the Klamath River basin are focused on the removal of dams from the mainstem river, relatively small investments in the restoration and management of high-priority headwater basins in the Klamath Mountains would help to insure the availability of high-quality refugia for aquatic species. Prioritization of restoration efforts could be based on spatially explicit models that predict susceptibility to debris flows and debris floods, combined with geographic data on previously disturbed streams and critical refugia for various species.

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Hierarchical Bayesian estimation of recruitment parameters and reference points for Pacific rockfishes (*Sebastes* spp.) under alternative assumptions about the stock–recruit function

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Abstract: Hierarchical Bayesian meta-analysis can be a useful method for improving estimation of key parameters for harvested fish populations. In hierarchical models, data from multiple populations are used simultaneously to obtain estimates of parameters for individual populations and characterize the variability among populations. Many populations of Pacific rockfishes (*Sebastes* spp.) have declined off the US West Coast since the 1980s, and there is also concern for their conservation in Canada. We develop a hierarchical Bayesian meta-analysis to improve estimates of stock–recruit parameters, characterize management-related parameters (e.g., optimal harvest rate), and address uncertainties in the structural form of the stock–recruit function for Pacific rockfishes. We estimate steepness and optimal harvest rates for 14 populations of Pacific rockfishes under alternative assumptions about the underlying stock–recruit function (Beverton–Holt and Ricker). We provide a posterior predictive distribution of steepness for rockfishes that can be used as a prior in future assessments for similar populations. We also evaluate whether $F_{40\%}$ is an appropriate proxy for F_{MSY} for Pacific rockfishes and show that uncertainty in the natural mortality rate can have a significant effect on management advice derived from meta-analyses of stock–recruit data.

Résumé : Une méta-analyse hiérarchique bayésienne peut être une méthode utile pour améliorer l'estimation des paramètres importants des populations de poissons exploitées. Dans les modèles hiérarchiques, on utilise simultanément des données provenant de plusieurs populations pour obtenir des estimations des paramètres pour les populations individuelles et caractériser la variabilité entre les populations. Plusieurs populations de sébastes (*Sebastes* spp.) du Pacifique connaissent un déclin depuis les années 1980 sur la côte occidentale des É.-U. et il y a aussi des inquiétudes au sujet de leur conservation au Canada. Nous avons mis au point une méta-analyse hiérarchique bayésienne pour améliorer l'estimation des paramètres de stock–recrutement, pour caractériser les paramètres reliés à la gestion (par ex., le taux optimal de récolte) et pour tenir compte de certaines incertitudes concernant la forme structurelle de la fonction stock–recrutement chez les sébastes du Pacifique. Nous estimons l'inclinaison et les taux optimaux de récolte de 14 populations de sébastes du Pacifique sous diverses présuppositions de rechange concernant les fonctions sous-jacentes de stock–recrutement (Beverton–Holt et Ricker). Nous fournissons une distribution prédictive a posteriori de l'inclinaison pour les sébastes qui peut servir de distribution a priori dans des évaluations futures de populations semblables. Nous vérifions aussi si $F_{40\%}$ peut servir de valeur de remplacement de F_{MSY} chez les sébastes du Pacifique et nous démontrons que l'incertitude dans le taux de mortalité naturelle peut avoir un effet significatif sur les recommandations de gestion tirées des méta-analyses des données de stock–recrutement.

[Traduit par la Rédaction]

Introduction

More than 70 species of Pacific rockfishes (*Sebastes* spp.) occur along the western coast of North America from the Gulf of California to the Bering Sea (Love et al. 2002). Approximately 39 of these species occur in waters off British Columbia (BC), Canada. Many populations have declined off the US West Coast since the 1980s (Ralston 1998), and there is also concern for their conservation in Canada. The Committee on the Status of Endangered Wildlife in Canada

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(COSEWIC) has assessed the populations of canary rockfish (*Sebastes pinniger*), quillback rockfish (*Sebastes maliger*), and bocaccio (*Sebastes paucispinis*) as "threatened" and rougheye rockfish (*Sebastes aleutianus*), darkblotched rockfish (*Sebastes crameri*), and yelloweye rockfish (*Sebastes ruberrimus*) as populations of "special concern" in British Columbian waters (COSEWIC, http://www.cosewic.gc.ca). Fisheries and Oceans Canada have implemented 164 rockfish conservation areas in BC to assist in the management of the shallower rockfish species, some of which are considered to have been overfished (Yamanaka and Logan 2010). With some exceptions, there is a general lack of reliable data for the stock assessment of Pacific rockfishes (Dorn 2002; Hilborn et al. 2002), and in Canada, formal assessments have only been completed for a few species (e.g., Richards and Olsen 1996; Schnute et al. 2001; Stanley et al. 2009).

In the event that a "threatened" status for a rockfish population is approved by the Federal Government of Canada, a recovery strategy would have to be implemented for the population, consistent with requirements of the Canadian Species at Risk Act (SARA). Development of recovery strategies for rockfish species in Canada will involve some use of predictive population models to evaluate performance of alternative management strategies. Performance can be measured in terms of certain metrics relative to agreed reference points, e.g., the ratio of current biomass (B) to biomass at maximum sustainable yield (MSY) (B_{MSY}) or current fishing mortality (F) relative to that which produces maximum sustainable yield (F_{MSY}).

In the US, the Pacific Fishery Management Council has implemented a management plan for Pacific rockfishes that is consistent with the Magnuson–Stevens Fishery Conservation and Management Act (Ralston 2002). The management plan states that fishing mortality should be kept below F_{MSY} and that fishing mortality should be reduced when B is below B_{MSY}. Although Canada has not legislated MSY as a management goal, it does require that fisheries be managed in a precautionary manner (Fisheries and Oceans Canada 2006; Shelton and Sinclair 2008). Therefore, estimates of sustainable fishing thresholds are required for any management plan. F_{MSY} (or its discrete equivalent, annual optimal exploitation rate, U_{MSY}) is widely considered a precautionary limit to fishing mortality that can prevent both growth and recruitment overfishing if successfully implemented (Punt 2000; Mace 2001; Punt and Smith 2001).

Estimation of productivity parameters

F_{MSY} is a function of the productivity of a fish population determined, at least in part, by the magnitude of density-dependent processes affecting the rate of survival of juveniles to recruitment age (Rose et al. 2001; Myers 2002; Walters and Martell 2004). The slope at the origin (α) of the Beverton–Holt (Beverton and Holt 1957) and Ricker (1954) stock–recruit functions describes the maximum recruitment potential in response to reductions in density of spawners due to fishing. This parameter (along with parameters describing the rates of growth and natural mortality and the age at recruitment to the fishery) is one of the principal determinants of F_{MSY}. Although α is an estimable, biologically meaningful parameter, it is not directly comparable among different populations, as it is sensitive to the units used to measure the spawning biomass. For example, when the independent axis of the stock–recruit function represents absolute numbers of eggs, α represents the maximum rate of survival from egg to recruit and has a maximum value of one. Usually, however, stock–recruit functions are parameterised in terms of spawning stock biomass, which can be measured in many different units. In these cases, α will be unit-dependent, representing the maximum number of surviving recruits per unit of biomass. To permit comparison of recruitment productivity among populations, stock–recruit functions are commonly reparameterised in terms of unitless functions of α. For example, steepness (h) is defined as the proportion of unfished recruits that are produced when spawning stock size is reduced to the arbitrary level of 20% of the unfished stock size (Mace and Doonan 1988). An alternative is the compensation ratio (CR), defined as the ratio of α to the slope of the line drawn between the origin and the point on the stock–recruit curve at which the stock is unfished (Goodyear 1977; Myers et al. 1999; for illustration, see Goodwin et al. 2006 (their fig. 1)).

Reliable estimation of recruitment parameters is dependent on long, contrasting time series of stock–recruit data (Hilborn and Walters 1992; Conn et al. 2010). However, informative data are often not available, leading some stock assessment scientists to simply fix h at an assumed value, despite its key role in determining F_{MSY} and other important reference points. Alternatively, Bayesian methods are often used to quantify the uncertainty surrounding estimates of steepness and other productivity parameters (McAllister et al. 1994; Punt and Hilborn 1997; Chen et al. 2003). If there is too little information in the data, however, posterior estimates of productivity parameters can be strongly influenced by the formulation of the prior probability density function (pdf). Despite recent advances in advice for formulating priors for productivity parameters (Myers et al. 1999, 2002; Mangel et al. 2010), in our experience, the appropriate range of values to admit for steepness (either as a fixed value or as a prior pdf) is one of the most contentious topics in any fisheries stock assessment (see also Punt and Hilborn 1997). Hierarchical Bayesian meta-analysis (Gelman et al. 1995) has been proposed as a method for improving estimation of recruitment productivity parameters when data are uninformative (Liermann and Hilborn 1997; Dorn 2002; Michielsens and McAllister 2004). In hierarchical Bayesian models, data from multiple populations are used simultaneously to obtain estimates of parameters for individual populations. Rather than pooling data, hierarchical models assume that there is a probability distribution for the parameter of interest (e.g., steepness) that is common to all populations. This common distribution is called the prior distribution. Each population is treated as an exchangeable unit for estimating the "hyper-parameters" of this prior distribution (e.g., its mean and standard deviation (SD)). The prior distribution for steepness acts as the prior pdf for each individual population. Multiple stock–recruit data sets are therefore used to jointly estimate the posterior pdfs of steepness for individual populations, while simultaneously estimating posterior pdfs for the hyper-parameters of the prior distribution (Gelman et al. 1995). The assumption of exchangeability of populations helps to reduce the problem of overfitting and enables more precise parameter estimation for populations for which

Table 1. List of parameters.

Parameter	Description (units, if applicable)
F	Instantaneous fishing mortality rate (year^{-1})
U	Annual exploitation rate ($U = 1 - e^{-F}$)
U_{MSY}	Optimal harvest rate
MSY	Maximum sustainable yield (t)
M	Instantaneous natural mortality rate (year^{-1})
h	Steepness
B	Biomass
SSB	Spawning stock biomass
SSB_{Max}	Maximum observed spawning stock biomass
VB	Vulnerable biomass
R	Recruits (numbers)
B	Population total biomass
α	Slope of the stock–recruit function at the origin
β	Scaling parameter of stock–recruit function
CR	Compensation ratio
φ_E	Equilibrium eggs per recruit
φ_B	Equilibrium total biomass per recruit
φ_{VB}	Equilibrium vulnerable biomass per recruit
SPR	Spawning stock biomass per recruit (equivalent to φ_E, see text)
$\%SPR_0$	Percentage of unfished SPR at a given harvest rate
a_T	Terminal age class (years)
a_{rec}	Age at first recruitment to the fishery (years)
a_{mat}	Approximate age at maturity (years)
m_a	Maturity at age
f_a	Fecundity at age
v_a	Vulnerability at age
w_a	Mean weight at age
$\boldsymbol{\theta}$	Vector of stock-specific estimated parameters
$\boldsymbol{\Phi}$	Vector of hyper-parameters
σ_h	Variance of errors
τ	Precision of errors (inverse of the variance of errors)
a	Shape parameter of Beta probability density function
b	Shape parameter of Beta probability density function
μ_h	Mean of the prior distribution of h
σ_h	Standard deviation of the prior distribution of h

the data are uninformative (Gelman et al. 1995), although it is important to note that biased results may result if the assumption of exchangeability is violated.

Dorn (2002) used hierarchical Bayesian meta-analysis to estimate steepness for 11 Pacific rockfish populations. One of the intended aims of the analysis was to quantify how steepness varies across populations. The study was the first of its kind for rockfishes and made use of data for rockfish populations from the US West Coast, BC, and Alaska. Compared with estimates obtained from independent individual analyses, the range of mean estimates of steepness for individual populations from the hierarchical analysis was shrunk towards the average steepness among populations (Dorn 2002).

Proxies for productivity: $\%SPR_0$

In the absence of reliable estimates of F_{MSY}, proxies based on more readily obtainable life history and selectivity data are often used. In US West Coast groundfish fisheries, $F_{35\%}$ was initially adopted as a proxy for F_{MSY} in 1992 (for the history of the decision to use $F_{35\%}$, see Clark 2002). $F_{35\%}$ represents the fishing mortality rate that would reduce spawning stock biomass per recruit (SPR) to 35% of its unfished state (SPR_0) (Gabriel et al. 1989; Clark 1991; Mace and Sissenwine 1993). The 35% adopted for West Coast groundfish was increased to 40% of SPR_0 in 1997 due to concerns about continuing declines of rockfish populations and further work by Clark (1993; see also Mace 1994). Clark (2002) showed that a much more conservative target fishing mortality rate of $F_{60\%}$ was robust to a wide range of uncertainty about productivity of the population, although Hilborn et al. (2002) argued that this may be too cautious for some populations, given uncertainty in natural mortality, and could lead to unnecessary risk of economic loss. Dorn (2002) tested the validity of the $F_{40\%}$ proxy, using the estimates of F_{MSY} derived from his estimates of steepness for 11 rockfish populations. His results suggested that fishing mortality rates in the range of $F_{40\%}$ to $F_{60\%}$ should be considered for data-limited West Coast rockfishes. This result, as well as other research on rockfish productivity, led to the adoption of $F_{50\%}$ as the default F_{MSY} proxy for rockfishes managed by Pacific Fishery Management Council. In contrast, the default F_{MSY} proxy used for rockfish populations in Alaskan water continues to be $F_{35\%}$ (although catch recommendations are based on $F_{40\%}$).

Current study

Despite improvements in the length of time series data for US Pacific rockfishes in recent years, many still lack sufficient contrast to reliably estimate recruitment parameters in individual assessments. Given the current need for estimates of productivity of various rockfishes in BC, it is timely to update the work of Dorn (2002). In this paper, we develop a new hierarchical Bayesian meta-analysis to estimate steepness and U_{MSY} for 14 populations of Pacific rockfishes under alternative assumptions about the underlying stock–recruit function (Beverton–Holt and Ricker). This work builds on the analysis of Dorn (2002), incorporating the original data for eight of his 11 populations, extended data for two of the original populations, and adding data for four "new" Alaskan populations. Data for two of the original Pacific ocean perch populations (Gulf of Alaska and Bering Sea) were combined for consistency with more recent data. Our results provide a posterior predictive distribution of steepness for rockfishes that could be used as a prior in future assessments for similar populations, as well as posterior pdfs of steepness for individual populations. We also update estimates of fisheries reference points for these species and re-evaluate whether $F_{40\%}$ is an appropriate proxy for F_{MSY} for Pacific rockfishes.

Materials and methods

Hierarchical models were developed to estimate parameters for 14 populations of Pacific rockfishes under alternative (Beverton–Holt and Ricker) assumptions about the underlying stock–recruit function. Model parameters referred to frequently in the text are defined in Table 1, the data are described in the next section, and details of the model follow.

Data

The models were fitted to stock–recruit data for 14 populations of Pacific rockfishes using the fixed parameters listed (Table 2) and fixed vulnerability, weight-at-age, and maturity-at-age schedules for each population, provided by the US National Marine Fisheries Service (NMFS; Martin Dorn, NMFS, Alaska Fisheries Science Center, Seattle, Washington).

Stock–recruit data sets were also provided by the Alaska Fisheries Science Center and came from a variety of stock assessments based on fisheries-independent surveys and other data published by the Pacific and North Pacific Management Councils, Fisheries and Oceans Canada, and the stock–recruit database of Myers et al. (1995; for further details, see the references in Table 2 and Dorn (2002)).

The stock–recruit data were all outputs from age- or length-structured stock assessments (Table 2). Care was taken not to use assessments that had predicted recruitment using an informative prior or fixed value for steepness (see Dorn 2002). In the original West Coast Pacific ocean perch assessment (Ianelli and Zimmerman 1998), a prior on steepness had been used, but for the purposes of generating data for this meta-analysis, the prior was turned off.

In the Alaskan assessments, recruitment was modelled as log mean recruitment plus an annual deviance. A likelihood penalty was used to constrain recruitment deviations but no explicit stock–recruit relationship was used. Though it could be argued that this implies a stock–recruit relationship with

Table 2. Life history and selectivity parameters for the 14 rockfish stocks in the analysis.

Common name	*Sebastes* spp.	Population	Population code	M	a_T	a_{50}	a_{sel}	Assessment model	N	Reference
Chilipepper rockfish	*S. goodei*	US West Coast[a]	Chilipepper	0.24	21	2	~4	SS–length	28	Rubinin et al. 1998
Bocaccio	*S. paucispinis*	US West Coast[a]	Bocaccio	0.2	21	1	~4.5	SS–length	30	MacCall et al. 1999
Widow rockfish	*S. entomelas*	US West Coast[a]	Widow	0.15	20	1	~6.5	SS–age	27	Ralston and Pearson 1997
Canary rockfish	*S. pinniger*	US West Coast[a]	Canary	0.08	25	3	~8	SS–age, ADMB–length	28	Crone et al. 1999; Williams et al. 1999
Black rockfish	*S. melanops*	WA, OR[a]	Black	0.28	16	6	~11	ADMB–age	7	Wallace et al. 1999
Yelloweail rockfish	*S. flavidus*	US West Coast[a]	Yelloweail	0.14	25	4	~10	ADMB–age	27	Tagart et al. 1997
Pacific ocean perch	*S. alutus*	US West Coast[a]	POP WC	0.06	25	3	~10	ADMB–age	40	Ianelli and Zimmerman 1998
		Goose Island Gully	POP GIG	0.05	30	7	~7	ADMB–age	26	Richards and Olsen 1996
		Gulf of Alaska[b]	POP GoA	0.06	25	2	~10	ADMB–age	43	Hanselman et al. 2007
		Bering Sea – Aleutian Islands[c]	POP BSAI	0.06	25	3	~11	ADMB–age	37	Spencer and Ianelli 2007*a*
Northern rockfish	*S. polyspinis*	Bering Sea – Aleutian Islands	Northern BSAI	0.04	23	3	~13	ADMB–age	20	Spencer and Ianelli 2007*b*
		Gulf of Alaska	Northern GoA	0.06	23	2	~13	ADMB–age	28	Heifetz et al. 2007
Dusky rockfish	*S. variabilis*	Gulf of Alaska	Dusky GoA	0.07	21	4	~11	ADMB–age	25	Lunsford et al. 2007
Rougheye rockfish	*S. aleutianus*	Gulf of Alaska	Rougheye GoA	0.03	25	3	~16	ADMB–age	26	Kaiser Shotwell et al. 2007

Note: Source: National Marine Fisheries Service, Alaska Fisheries Science Center, Seattle, Washington (M. Dorn). M, average instantaneous natural mortality rate; a_T, terminal age (note that this may be considerably younger than the population's maximum age); a_{50}, age at first recruitment, i.e., youngest observed age in fishery; a_{sel}, age at 50% maturity; N, number of years of stock–recruit observations; SS-age, Stock Synthesis (Methot 1989), age-based model; SS-length, Stock Synthesis length-based model; ADMB-age, AD Model Builder (Otter Research 1996), age-structured model; and ADMB-length, AD Model Builder, size-structured model.

[a]Data same as used by Dorn 2002.

[b]Data updated since Dorn 2002.

Table 3. Derivation of recruitment parameters for the alternative models.

Beverton–Holt		Ricker	
Eq. no.	Equation	Eq. no.	Equation
T3.1*a*[a]	$\mathrm{CR} = \frac{4h}{1-h}$	T3.1*b*[b]	$\mathrm{CR} = (5h)^{5/4}$
T3.2*a*	$\alpha_1 = \frac{\mathrm{CR}}{\varphi_{E_0}}$	T3.2*b*	$\alpha_2 = \frac{\mathrm{CR}}{\varphi_{E_0}}$
T3.3*a*	$R_0 = \frac{B_0}{\varphi_{B_0}}$	T3.3*b*	$R_0 = \frac{B_0}{\varphi_{B_0}}$
T3.4*a*	$\beta_1 = \frac{\alpha_1\varphi_{B_0} - 1}{R_0\varphi_{B_0}}$	T3.4*b*	$\beta_2 = \frac{\ln(\alpha_2\varphi_{B_0})}{R_0\varphi_{E_0}}$
T3.5*a*[c]	$R_U = \frac{\alpha_1\varphi_{EU} - 1}{\beta_1\varphi_{EU}}$	T3.5*b*	$R_U = \frac{\ln(\alpha_2\varphi_{EU})}{\beta_2\varphi_{EU}}$
T3.6[d]	$\mathrm{MSY} = \varphi_{VB U_{MSY}} R_{MSY} U_{MSY}$		
T3.7[e]	$\mathrm{SSB}_{MSY} = R_{MSY}\varphi_{EU_{MSY}}$		
T3.8	$\mathrm{SSB}_0 = R_0\varphi_{E_0}$		

Note: Subscript 0 indicates $U = 0$ and subscript U indicates that the parameter has been calculated with the tested value of U in the survivorship function (eq. T4.2).

[a]Myers et al. 1999.

[b]Michielsens and McAllister 2004.

[c]This function returns R_{MSY} at U_{MSY}.

[d]MSY and U_{MSY} obtained through numerical search (see eq. 5); see Table 4 for definition of $\varphi_{VB U_{MSY}}$.

[e]$\varphi_{EU_{MSY}}$ obtained using eq. T4.3, with l_a evaluated at U_{MSY}.

a steepness of one, this approach is probably the least likely to influence the results of the present analysis, given the alternatives for setting up stock assessment models. The US West Coast assessments were done using the assessment model Stock Synthesis (Methot 1989; Table 2). Generally, the recruitment time series in these models were estimated as a set of free parameters but could be constrained to follow a stock–recruit relationship depending on the variance assumed. Most of the assessments put very low emphasis on the stock–recruit likelihood component. For example, in the widow rockfish assessment, model runs were structured so that the emphasis placed on the stock–recruit component of the model was quite low, thus preventing the stock–recruit function from having undue influence on the estimation of year-specific recruitment (Ralston and Pearson 1997).

Model description

Two of the most widely used stock–recruit functions are the Beverton–Holt (eq. 1) and Ricker (eq. 2) models, i.e.,

$$(1) \qquad R = \frac{\alpha_1 E}{1 + \beta_1 E}$$

$$(2) \qquad R = \alpha_2 E e^{-\beta_2 E}$$

where R is the number of recruits and E is the number of eggs. Equations 1 and 2 have parameters α and β, describing productivity and scale, respectively (with subscripts 1 and 2 representing parameters of the Beverton–Holt and Ricker models, respectively). In these formulations, parameter α describes the slope of the function close to the origin (i.e., the maximum rate of survival from egg to recruit if the independent variable is absolute numbers of eggs) and is equivalent for both stock–recruit functions (Myers et al. 1999), although the two functions tend to result in different estimates of α if fitted to the same data (Walters and Martell 2004). Hilborn and Walters (1992) provide a detailed interpretation of β for the two functions. In our analysis, the number of eggs was assumed to be proportional to spawning stock biomass, where fecundity at age (f_a) was given by

$$(3) \qquad f_a = m_a w_a$$

where m_a is the proportion of females mature at age a and w_a is mean weight at age a of females.

Here we define a recruit as a fish of the youngest age observed in the fishery, even if that age class is not fully vulnerable to the fishing gear (ages at recruitment, a_{rec}, were assumed to be constant and are provided in Table 2). Vulnerability at age was assumed fixed over time, according to vulnerability schedules provided by the National Marine Fisheries Service (NMFS). Vulnerability schedules were described by saturating logistic functions for all populations except bocaccio, widow rockfish, yellowtail rockfish, and West Coast Pacific ocean perch, for which vulnerabilities were described by dome-shaped curves.

Models were constructed so that key estimated parameters (leading parameters) were steepness (h) and unfished total biomass (B_0). Steps to transforming h and B_0 to recruitment parameters α and β are provided (Table 3). We first show the relationship between h and the compensation ratio (CR) (eqs. T3.1*a* and T3.1*b*) and then the relationship between CR and α (eqs. T3.2*a* and T3.2*b*). CR is the ratio of α to the equilibrium unfished slope of the stock–recruit function, i.e., R_0/E_0, where R_0 is the unfished recruitment and E_0 is the unfished egg production (see Goodwin et al. 2006, their fig. 1). Equilibrium unfished eggs per recruit (φ_{E_0}) and equilibrium unfished total biomass per recruit (φ_{B_0}), used in eqs. T3.2 and T3.3, were obtained using the functions given in Table 4 (eqs. T4.3 and T4.4), with the harvest rate U set to zero in the survivorship function (eq. T4.2). We used a plus group in our calculations of survivorship for the terminal age class (eq. T4.2), as terminal age (a_T) was considerably lower than the maximum age for most populations. We could have combined eqs. T3.1*a* and T3.2*a* (and eqs. T3.1*b* and T3.2*b*), as in Michielsens and McAllister (2004), but

Table 4. Variables common to all models.

Eq. no.	Variable
Natural survival rate at age[a]	
T4.1	$s_a = e^{-M_a}$
Survivorship at age	
T4.2	$l_a = \begin{cases} 1 & \text{if } a = a_{min} \\ \ell_{a-1}s_{a-1}(1 - v_{a-1}U) & \text{if } a > a_{min} \\ \dfrac{\ell_{a-1}s_{a-1}(1 - v_{a-1}U)}{(1 - S_{m_T})} & \text{if } a = a_T \end{cases}$
Incidence functions[b]	
T4.3	Equilibrium eggs per recruit at U: $\varphi_{EU} = \sum_{a=a_{min}}^{\infty} l_a f_a$
T4.4	Equilibrium total biomass per recruit at U: $\varphi_{BU} = \sum_{a=a_{min}}^{\infty} l_a w_a$
T4.5	Equilibrium vulnerable biomass per recruit at U: $\varphi_{VBU} = \sum_{a=a_{min}}^{\infty} l_a w_a v_a$

Note: M, average instantaneous natural mortality rate (assumed constant for all age classes); a_T, terminal age (maximum age observed in fishery); S_{m_T}, survival rate at terminal age; a_{min}, youngest observed age in fishery; v_a, vulnerability at age; f_a, fecundity at age; w_a, weight at age; U, annual harvest rate.

[a]Note that M_a was fixed in all cases except three populations in scenario S4 (see text and Fig. 1).
[b]Botsford 1981.

have chosen to show CR as a separate function to illustrate the analytical linkages between these three important productivity parameters. Note that our eq. T3.2*a* is the inverse of eq. 10 of Michielsens and McAllister (2004) because of the different formulation of the Beverton–Holt function.

The relationship between α, CR, and φ_{E_0} is the same for both stock–recruit models (eqs. T3.2*a* and T3.2*b*). Because $\varphi_{E_0} = \frac{E_0}{R_0}$, the equilibrium unfished slope of the stock–recruit function is simply $(\varphi_{E_0})^{-1}$ and $CR = \frac{\alpha}{(\varphi_{E_0})^{-1}}$ or $\alpha\varphi_{E_0}$ (Goodyear 1977; Myers et al. 1999).

Under steady-state conditions, eqs. 1 and 2 can be reparameterised in terms of equilibrium eggs per recruit φ_E, which provides a convenient means of initialising dynamic age-structured models (Botsford 1981). This is done by substituting $E = R\varphi_E$ into eqs. 1 and 2 and re-arranging to obtain

$$(4) \qquad R = \frac{\varphi_E\alpha_1 - 1}{\beta_1\varphi_E}$$

and

$$(5) \qquad R = \frac{\ln(\alpha_2\varphi_E)}{\beta_2\varphi_E}$$

for the Beverton–Holt and Ricker functions, respectively (Walters and Martell 2004). After obtaining α (eqs. T3.2*a* and T3.2*b*), eqs. 4 and 5 can be rearranged to obtain β (eqs. T3.4*a* and T3.4*b*), completing the steps needed to obtain recruitment parameters from the key estimated parameters.

Following estimation of h and B_0, U_{MSY} and MSY can be obtained numerically by iteratively searching for the value of U that maximizes long-term yield Y given by

$$(6) \qquad Y = UR\varphi_{VB}$$

where R is equilibrium recruitment under the harvest rate U (eqs. T3.5*a* and T3.5*b*) and φ_{VB} is equilibrium vulnerable biomass per recruit under U (eq. T4.5, with survivorship evaluated at the tested value of U). Once U_{MSY} has been found by numerical search, eq. 6 can be written more specifically, as in eq. T3.6. It is then possible to calculate the spawning stock biomass at MSY (SSB_{MSY}; eq. T3.7), unfished spawning stock biomass (SSB_0; eq. T3.8), and the fishery reference point B_{MSY}/B_0 (i.e., SSB_{MSY}/SSB_0).

Note that under our assumption that fecundity is proportional to female weight at age (eq. 3), φ_E is the equivalent of spawning stock biomass per recruit (i.e., $SPR = \sum_{a=a_{min}}^{\infty} l_a w_a m_a$, where l_a is survivorship at age (eq. T4.2; Gabriel et al. 1989). Equilibrium SPR at harvest rate U (SPR_U) is therefore equivalent to φ_{E_U} (eq. T4.3). The reference point %SPR_0 at U_{MSY} for each population was therefore calculated as the ratio $\varphi_{EU_{MSY}}/\varphi_{E_0}$, where $\varphi_{EU_{MSY}}$ was calculated using the mean of the posterior pdf of U_{MSY} for each population.

Hierarchical Bayesian models

In the hierarchical Bayesian model structure, probability distributions link the data, X, to parameters, $\boldsymbol{\Theta}$, and the parameters to hyper-parameters, $\boldsymbol{\Phi}$, with the prior joint distribution of the parameters and hyper-parameters given by

$$(7) \qquad p(\boldsymbol{\Theta}, \boldsymbol{\Phi}) = p(\boldsymbol{\Theta}|\boldsymbol{\Phi})p(\boldsymbol{\Phi})$$

and the joint posterior distribution given, according to Bayes' rule, by

$$(8) \qquad p(\boldsymbol{\Theta}, \boldsymbol{\Phi}|X) \propto p(X|\boldsymbol{\Theta})p(\boldsymbol{\Theta}|\boldsymbol{\Phi})p(\boldsymbol{\Phi})$$

(Gelman et al. 1995; Dorn 2002). In the present analysis, the hierarchical Bayesian models had estimated hyper-parameters ($\boldsymbol{\Phi}$, eq. T5.1; Table 5), estimated population-specific parameters ($\boldsymbol{\Theta}$, eq. T5.2; Table 5), and fixed population-specific life history and selectivity parameters ($\boldsymbol{\theta}$, eq. T5.3; Table 5). For tractability, life history and selectivity parameters for the 14 populations were assumed to be fixed and known

Table 5. Vectors of estimated and fixed parameters for the hierarchical models for $k = 14$ populations (see Table 1 for key to parameter names).

Eq. no.	Vector
Hyper-parameters	
T5.1*a*	$\boldsymbol{\Phi} = (\mu_h, \sigma_h)$
Individual stock parameters	
T5.2*a*	$\boldsymbol{\Theta}_k = (h_k, B_{0k}, \tau_k)$
Individual stock parameters (fixed, see Table 2 for values)	
T5.3	$\boldsymbol{\theta}_k = (M_k, \sigma_{Tk}, a_{mat\,k})$

perfectly (Table 2). Sensitivity to the assumed values of M was tested (see below).

Likelihood $\mathbf{p(X|\Theta)}$

We used a lognormal density function for the likelihood. The negative log likelihood function for K populations is as follows:

$$(9) \qquad -\ln L = \sum_{k=1}^{K}\left(n_k \ln(\sigma_k) + \sum_{i=1}^{n_k}\frac{{d_{ki}}^2}{2{\sigma_k}^2}\right) + \text{constant}$$

where d_{ki} is the difference between the log of observed recruits and the log of predicted recruits given $(\boldsymbol{\Theta}, \boldsymbol{\Phi})$ for the kth population and ith observation, assuming either Beverton–Holt or Ricker recruitment, and n_k is the number of observations for each population k. σ_k describes the SD of the residuals (i.e., process error) for the kth population and is derived from the estimated precision parameter τ, which is the inverse of the variance of the residuals (i.e., $\sigma_k = \sqrt{1/\tau_k}$). For simplicity, we assumed that there was no autocorrelation in recruitment, a common simplifying assumption.

Nonhierarchical prior $\mathbf{p(\Theta|\Phi)}$

For each population, equilibrium unfished total biomass (B_0) was assigned a broad uniform prior with a lower limit of 10% of the maximum observed spawning biomass ($0.1\,SSB_{Max}$) and an upper limit of 10 times the maximum observed spawning biomass ($10\,SSB_{Max}$). The upper boundary was determined during trial runs by inspecting scatterplots of posterior values of steepness vs. B_0 (Appendix A, Fig. A1) to ensure that most of the posterior probability density was captured in the analysis (i.e., to ensure that there were no significant boundary problems in the MCMC search). As noted by Dorn (2002), who used the data similarly to guide setting of his prior for R_0, this approach is not strictly within Bayesian principles as it involves using the data twice, but it was necessary to place some boundary on the prior and to curb the tendency of the models to sample extremely large values of B_0, especially for populations with uninformative data. This upper boundary was sufficient to capture almost all of the posterior probability density for all but a few populations (notably the Bering Sea – Aleutian Islands population of northern rockfish and West Coast Pacific ocean perch), which exhibited extremely long right-hand tails of the posterior probability density for B_0 (Appendix A). Sensitivity to the effects of the upper boundary placed on this prior was tested (see below).

In both models, we followed the approach of Michielsens and McAllister (2004) and defined the prior for the precision of the recruitment deviations (τ, the inverse of the variance) as an uninformative gamma distribution (Gelman et al. 1995), i.e., $\tau \sim \Gamma(0.001, 0.001)$.

Hierarchical parameters and hyper-prior $\mathbf{p(\Phi)}$

In a hierarchical model, one or more parameters (h, in this case) are assumed to come from a probability distribution shared by all populations (the prior distribution). The hyper-parameters that describe the prior distribution for h (the hyper-priors; eq. T5.1) are estimated along with parameters for individual populations (eq. T5.2).

The prior distribution for h was assumed to conform to a transformed Beta distribution. The Beta distribution is defined on the interval $(0, 1]$, with its shape determined by two positive parameters, a and b (Gelman et al. 1995). Under Beverton–Holt recruitment, however, h is defined on the interval $[0.2, 1)$. It was therefore necessary to transform the prior, i.e.,

$$(10) \qquad h = 0.8\,\text{Beta}(a, b) + 0.2$$

Under Ricker recruitment, it is possible for recruitment at 20% unfished stock size to be greater than unfished recruitment and, therefore, for h to take values greater than 1. Although the theoretical limit to h under Ricker recruitment is infinity, there appear to be natural constraints on its value (Winemiller 2005; Mangel et al. 2010). Myers et al. (1999) did not report mean values of CR greater than 123.5 for the 700 populations they analysed, which would correspond to a value of $h = 9.4$ under Ricker recruitment (eq. T3.1*b*). We consider an upper boundary of 6.0 for the uniform prior distribution of h to adequately contain the range of values that might be possible for rockfishes with Ricker recruitment. This corresponds to a CR of approximately 70.2, which was close to the upper limit of all values reported in Myers et al. (1999) — only two species had a higher mean CR. Similarly, Goodwin et al. (2006) reported CR less than 70 for all except five out of 54 populations they analysed.

To convert the value drawn from the prior Beta probability distribution to the range $[0.2, 6]$, the transformation is therefore

$$(11) \qquad h = 5.8\,\text{Beta}(a, b) + 0.2$$

The hyper-priors a and b can be derived from the Beta distribution's mean and SD (Gelman et al. 1995). Mean and SD are more easily interpreted than a and b, and it is therefore more straightforward to devise appropriate priors for these parameters. We therefore directly estimated the hyper-priors μ_h and σ_h, representing the mean and SD of the Beta distribution, and then transformed them to a and b (Gelman et al. 1995). Priors for μ_h and σ_h were set to reflect ignorance of the distribution of h. To obtain an uninformative prior distribution for h, we followed the approach of Michielsens and McAllister (2004). First, the prior for μ_h was drawn from an uninformative Beta distribution (Beta(2, 2)). This is not a completely uniform prior, as it places slightly less probability at the unlikely extreme ends of the distribution (Myers et al. 1999; Michielsens and McAllister 2004). Given the constraints on the range of the Beta distribution, it was necessary to constrain the range of σ_h for each sampled μ_h so that

the drawn value stayed between 0 and 1. For any given value of μ_h, assuming a symmetrical distribution of the Beta function, the maximum possible variance, $\max(\sigma^2)$, is given by

$$\max(\sigma^2) = \frac{(h_{max} - h_{min})^2}{12} \quad (12)$$

(Evans et al. 2000), where h_{max} is the upper limit of the range of steepness and h_{min} is the symmetrical lower limit, corresponding to the value of h that has been drawn (e.g., under Beverton–Holt recruitment, if a value of 0.8 has been drawn, h_{max} would be 1, h_{min} would be 0.6, and the corresponding maximum SD from eq. 12 would be ~0.12). We assumed that the maximum SD was the most likely value for σ_h, with a linear reduction in probability as σ_h approaches zero (Michielsens and McAllister 2004). The prior for σ_h was therefore obtained from

$$\sigma_h = \sqrt{\max(\sigma^2)} \cdot \text{Beta}(2, 1) \quad (13)$$

(for figures and WinBUGS code, see Michielsens and McAllister 2004). The hyper-priors were then transformed to the a and b parameters of the Beta distribution (Gelman et al. 1995), which were used to derive priors for h for each population (eqs. 10 and 11). The steps above resulted in uninformative steepness priors for each population.

Posterior $p(\Theta,\Phi|X)$

The log joint posterior probability distribution of the hierarchical model is proportional to the sum of the log likelihood, the log prior, and the log hyper-prior (Dorn 2002) (eq. 8). Posteriors were obtained using the Gibbs sampler version of the Markov chain Monte Carlo (MCMC) algorithm implemented using WinBUGS software (Bayesian inference using Gibbs sampling; Lunn et al. 2000; available from www.mrc-bsu.cam.ac.uk/bugs/). For each hierarchical model, we ran the MCMC for 250 000 iterations, discarding the first 200 000 to remove any "burn-in" effects. We initialised the chains from three different starting points and used the Gelman–Rubin diagnostic tool (Brooks and Gelman 1998), within the WinBUGS software, to ensure that the results had not been influenced by initial conditions. We examined trace plots of the Markov chain to make sure that chains had converged and used the autocorrelation tool within WinBUGS to ensure that the values used to produce posterior estimates were not autocorrelated. Posterior values were imported into the R programming environment (R Development Core Team 2006; available from www.r-project.org), using the CODA package for MCMC output. All further analysis was done in the R environment.

Sensitivity analyses

Seven sensitivity analyses were done to test the effects of (*i*) ranges of some priors, (*ii*) fixed input parameters (M), and (*iii*) individual data sets on posterior results. Sensitivity analyses were done using the hierarchical Beverton–Holt model only.

Sensitivity to ranges of nonhierarchical priors

In any Bayesian analysis, it is possible for estimated parameters to be confounded. This is a well-known problem in population dynamics modelling, where scale and productivity parameters are often highly confounded, especially when there is little contrast in the data (Hilborn and Walters 1992). Therefore, even if priors are completely uninformative (i.e., uniform), the upper and lower limits set on the prior for one parameter may affect the posterior of other parameters. In the present analysis, our prior for h for each population was uniform and bounded by its natural limits of 0.2 and 1 in the Beverton–Holt case and by 0.2 and 6 in the Ricker case (see above for justification of this upper limit). As these priors were uniform and naturally bounded, we did not do further sensitivity tests.

The priors for R_0, although uniform, were not naturally bounded. As discussed previously, preliminary tests showed that most of the posterior probability density was captured using upper and lower bounds for the R_0 priors of $0.1\,SSB_{Max}$ and $10\,SSB_{Max}$, respectively, where SSB_{Max} is the maximum observed spawning stock biomass for each population. However, there were some populations for which the posterior distributions of R_0 had very long right-hand tails (Appendix A, Fig. A1). Two sensitivity analyses were done to assess the impact of the range of the R_0 prior on posterior estimates of steepness. In the first (scenario S1), the upper limit of the prior was set to $20\,SSB_{Max}$ for each population (scatterplots of posterior samples of h and R_0 are shown in Appendix A, Fig. A2). For some populations for which $10\,SSB_{Max}$ was already sufficient to capture most of the posterior probability density, setting such a large prior resulted in instability in the posterior pdf for h (e.g., spikes of high density at improbably low values around 0.2). Therefore, in a second sensitivity analysis (scenario S2), the upper limit of the prior was varied for each population. The upper limit ($x\,SSB_{Max}$) was set by visual inspection of scatterplots of posterior samples of h and R_0 (Appendix A, Fig. A2) so that most of the posterior probability density was captured for both parameters, with x = 5, 10, 15, or 20. These cut-off points were arbitrary and somewhat subjective but represented an attempt to reach a compromise between bounding the prior too tightly on one hand and sampling too far at the extremes of the posterior density on the other. We made the assumption that 20 times the maximum observed spawning stock biomass was a sufficiently large upper limit to test and that values greater than this could be considered unlikely.

We also tested sensitivity to the range of the prior used for the precision parameter τ. In this run (scenario S3), the parameters of the gamma distribution were adjusted to give $\tau \sim \Gamma(0.0001, 0.0001)$.

Sensitivity to M

The rate of natural mortality (M) is an important determinant of steepness (Tables 3 and 4). This parameter is difficult to estimate, and for convenience, it is usually assumed to have some fixed value. The present analysis used constant values of M that had been used in previous stock assessments (Table 2; Dorn 2002). For 11 of the populations, M had been assumed fixed across all ages in previous analyses, and these values were used here. For the other three populations (canary rockfish, black rockfish, and yellowtail rockfish), M had been assumed previously to be an increasing function of age (Fig. 1). This is often done in stock assessments to help explain lower than expected observations of

old females. In our baseline analysis, we had assumed an average fixed value of M for canary, black, and yellowtail rockfishes constant across age classes, as reported in Dorn (2002). We tested sensitivity to this assumption by running the model again (scenario S4), incorporating the age schedules of M shown (Fig. 1) for these three populations. We ran two other sensitivity tests to broadly capture the effect of under- or over-estimating M. In the first (scenario S5), M was increased by 20% of its baseline value for all populations. In the second (scenario S6), M was decreased by 20% of its baseline value for all populations.

Effect of excluding individual data sets

To test the assumption of exchangeability among populations and to test whether the posterior predictive distribution of h was sensitive to any one particular data set, the Beverton–Holt hierarchical analysis was run a further 14 times, while systematically excluding one data set in each run.

Results

Posterior estimates were insensitive to initial conditions in all models, and all chains converged. There was little to no autocorrelation in the posterior MCMC chains for any model. It was not our aim to apply formal model-evaluation approaches to evaluate the credibility of alternatives (e.g., computation of Bayes factors; Gelman et al. 1995) and we present the results from the alternative stock–recruit functions as equally likely hypotheses (see Brodziak 2002; Dorn 2002). Fits to the stock–recruit data for both Beverton–Holt and Ricker models are shown (Fig. 2). These were generated using median posterior values of α and β for each population (eqs. T3.2 and T3.4). Many of the data sets had poor contrast, indicated by few or no data points at low population size. Notable exceptions were canary rockfish and West Coast Pacific ocean perch.

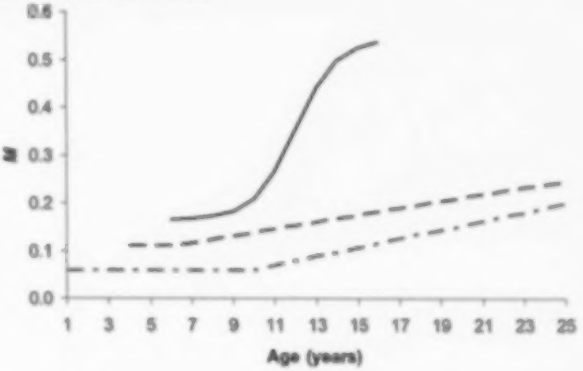
Effects of hierarchical structure on posterior estimates of steepness

Under the assumption of Beverton–Holt recruitment, there was some contraction in the range of median marginal posterior estimates of h from the hierarchical model compared with the independent models, although the change was minor for most populations (Fig. 3*a*). Populations for which the difference was greatest were chilipepper rockfish, bocaccio, widow rockfish, and Pacific ocean perch (Goose Island Gully), for which the independent models estimated lower h than the hierarchical model. Under Ricker recruitment, the contraction in overall range of median posterior estimates of h was greater, although this was driven mainly by the decrease in the estimate for chilipepper rockfish (Fig. 3*b*).

Posterior probability distributions for h

Marginal posterior probability distributions of h were obtained from the hierarchical models (Fig. 4; Table 6). The posterior predictive distribution (i.e., the posterior pdf for steepness common to all populations) was obtained by approximating integration across the posterior densities of hyper-parameters μ_h and σ_h. This was done by randomly drawing 10 000 pairs of values of μ_h and σ_h from their joint posterior distribution. Each of these 10 000 joint pairs described one posterior Beta distribution for h (after transformation using eq. 10 or eq. 11), from which a random value of h was drawn. The probability density of the resulting 10 000 values of h approximated the posterior predictive distribution of h. Under the assumption of Beverton–Holt recruitment, the posterior mean predictive estimate of h was 0.67, with median 0.69 and SD 0.17 (Fig. 4*a*). Under the assumption of Ricker recruitment, the posterior mean predictive estimate of h was 0.87, with median 0.80 and SD 0.38 (Fig. 4*b*).

Fig. 1. Age schedules of M used in sensitivity analysis S4 for the canary (dotted–dashed line), black (continuous line), and yellowtail (broken line) rockfish populations. M for all other populations was fixed across age classes.



Management parameters

U_{MSY} *and* B_{MSY}/B_0

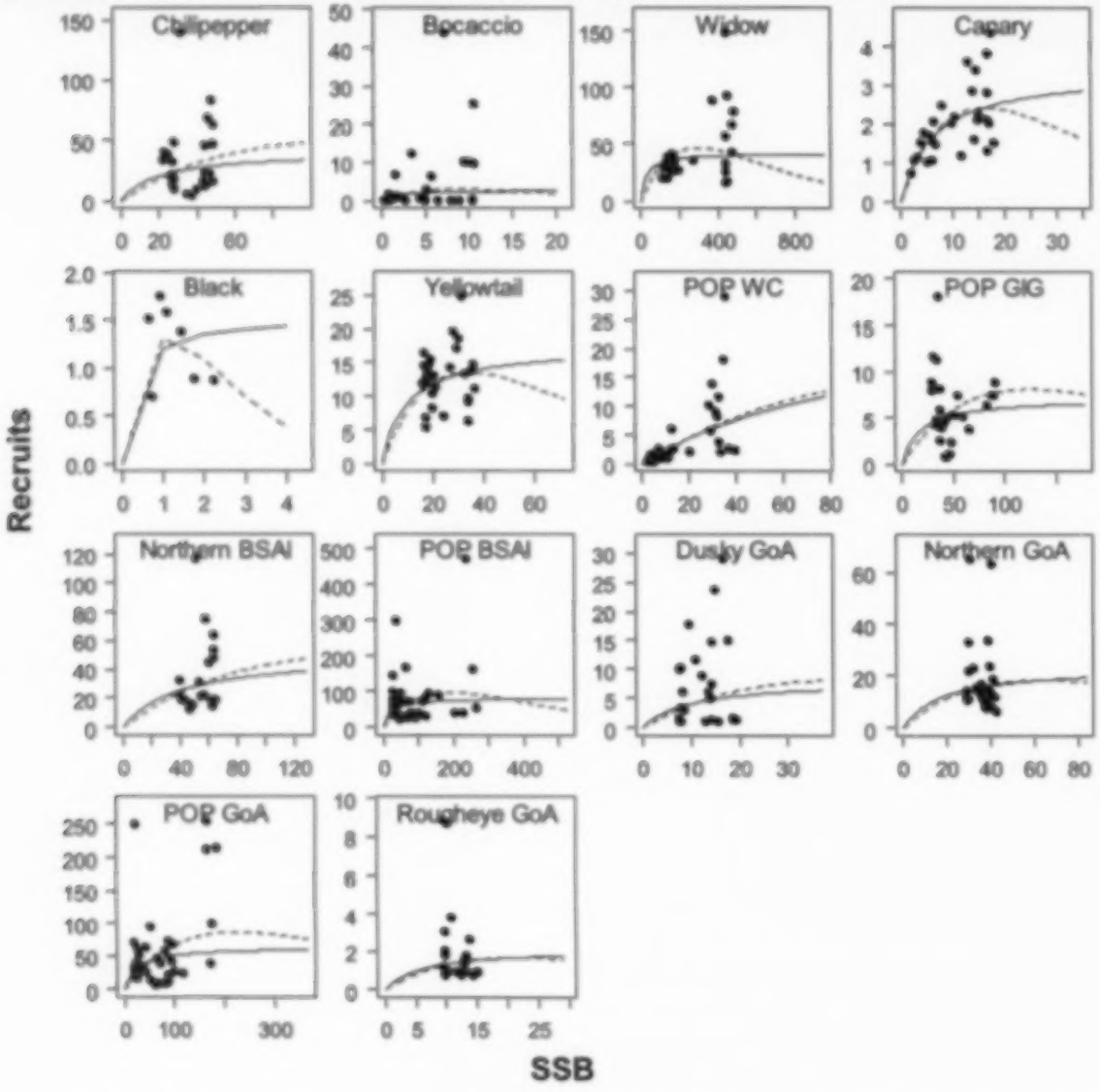
Marginal posterior distributions for optimal harvest rate U_{MSY} (Fig. 5; Table 6) were numerically derived from the posterior estimates of steepness using a Newton–Raphson algorithm. In general, mean values were relatively low (<0.2 in 12 cases and <0.1 in five cases) and posteriors were reasonably precise, although notable exceptions were the chilipepper rockfish and black rockfish populations (Fig. 5; Table 6). Populations with higher mean steepness did not necessarily have higher mean U_{MSY} (Fig. 6). This was the case under both stock–recruit assumptions and reflects that the relationship between these two parameters is unique to individual populations. This relationship is highly nonlinear and is determined by the population's life history and the selectivity schedule imposed upon it by the fishery (Forrest et al. 2008).

Under the assumption of Beverton–Holt recruitment, mean estimates of B_{MSY}/B_0 ranged from 0.19 to 0.38 (Fig. 7). Mean estimates of B_{MSY}/B_0 were higher under the assumption of Ricker recruitment, ranging from 0.36 to 0.47 (Fig. 7). Under Ricker recruitment, some estimates of B_{MSY}/B_0 were higher than mean estimates of $\%SPR_0$ at U_{MSY} (Table 6), which can happen if fish are first harvested before they mature, as was the case with many of the populations in this study.

$\%SPR_0$ *at* U_{MSY}

Posterior estimates of $\%SPR_0$ at mean U_{MSY} ranged from 0.21 to 0.58 under Beverton–Holt recruitment and from 0.22 to 0.76 under Ricker recruitment (Table 6). Based on these mean estimates, the proxy used by the North Pacific Fishery

Fig. 2. Fits to stock–recruit data obtained for the 14 stocks under the assumption of Beverton–Holt (continuous lines) and Ricker (broken lines) stock–recruit functions. Units vary among populations. See Table 2 for population codes.



Management Council, $F_{40\%}$, would serve as an adequate precautionary reference point for most of the populations if the stock–recruit relationship could be described by the Beverton–Holt function but would be inadequate for some populations (notably bocaccio and West Coast Pacific ocean perch). It would be an inadequate limit reference point for five populations under a Ricker stock–recruit relationship (Table 6).

Some other observations are worth noting, mainly that the six Alaskan populations all had low $\%SPR_0$ at U_{MSY} (21%–34%), despite having very low M (0.03–0.07 year^{-1}) (Table 6). This is less surprising when it is noted that these populations also tended to have higher estimated steepness (Table 6). For the 14 populations, there was a strong negative relationship between h and $\%SPR_0$ at U_{MSY} under both Beverton–Holt (r = –0.96) and Ricker (r = –0.94) recruitment (Figs. 8*a*, 8*b*). This might be expected, as populations with stronger recruitment compensation are expected to be able to be sustained at lower levels of spawning biomass. Several populations with high estimated steepness had very low M (Figs. 8*c*, 8*d*) and correspondingly low mean estimated U_{MSY} (Pacific ocean perch and Alaskan populations; Table 6). Therefore, although the relationship between $\%SPR_0$ at U_{MSY} and U_{MSY} was negative (r = –0.32 and –0.49 for Beverton–Holt and Ricker recruitment, respectively; Figs. 8*e*, 8*f*), as might be expected, the relationship was nowhere near as strong as that between $\%SPR_0$ and h. In other words, for many of these populations, M was a

Fig. 3. Median population-specific posterior estimates of h from (*a*) Beverton–Holt and (*b*) Ricker models. Independently derived estimates are shown on the left-hand side of each plot. Estimates derived from hierarchical models are shown on the right. Key to populations (see also Table 2): (a) chilipepper; (b) bocaccio; (c) widow; (d) canary; (e) black; (f) yellowtail; (g) POP WC; (h) POP GIG; (i) northern BSAI; (j) POP BSAI; (k) dusky GoA; (l) northern GoA; (m) POP GoA; and (n) rougheye GoA. See Table 2 for population codes.

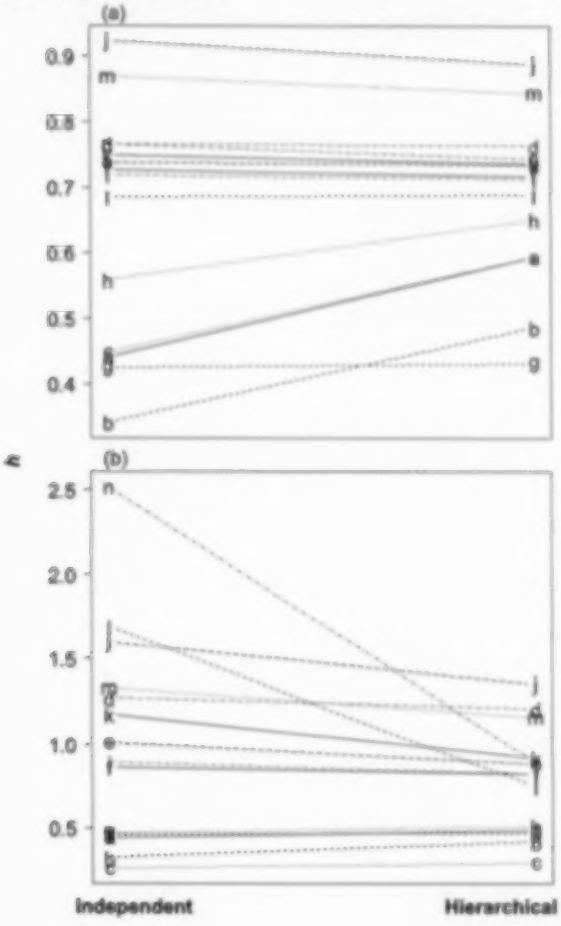
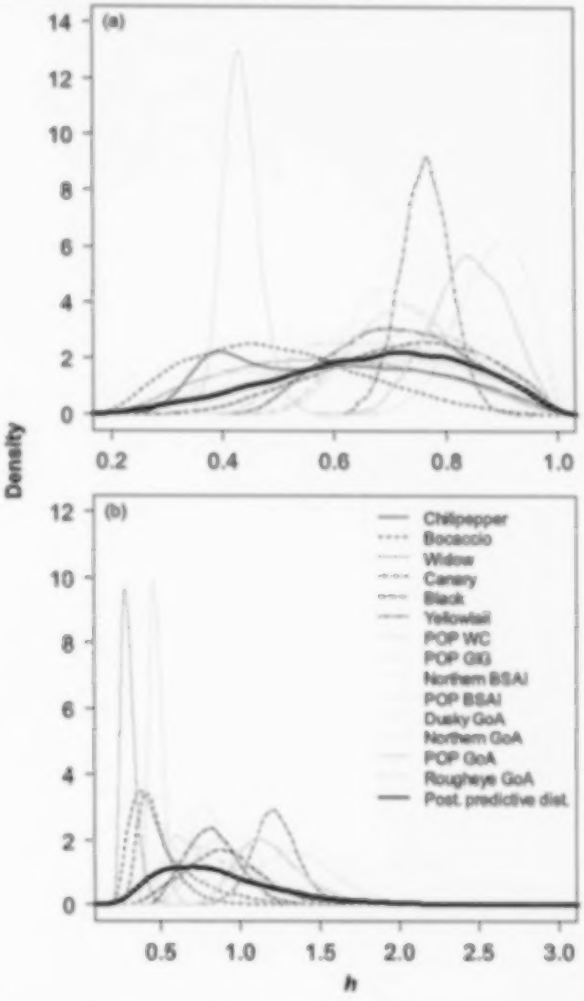


Fig. 4. Marginal posterior probability distributions of h obtained directly from the hierarchical models for the 14 stocks under (*a*) Beverton–Holt and (*b*) Ricker recruitment. The posterior predictive distribution (post. predictive dist.) for h is also shown (thick black line). See Table 2 for population codes.



stronger determinant of U_{MSY}, whereas steepness was the strongest determinant of $\%SPR_0$ at U_{MSY}. These two negatively related productivity parameters trade off against each other to produce a much flatter relationship between $\%SPR_0$ at U_{MSY} and U_{MSY} than might be expected. This is an encouraging result in terms of the suitability of $\%SPR_0$ at U_{MSY} as a proxy for U_{MSY}, as it remains relatively stable across a range of estimates of U_{MSY}.

Sensitivity analyses

Sensitivity to ranges of nonhierarchical priors

Increasing the range of values included in the prior distributions for B_0 had a noticeable effect on the mean of the posterior distribution of steepness for some populations but not others (Table 7). Not surprisingly, steepness posteriors for populations with the most informative data sets (canary rockfish, West Coast Pacific ocean perch, and Bering Sea – Aleutian Island Pacific ocean perch) were almost unaffected by changing the prior for B_0 (scenario S1). For a number of other populations, however, the mean estimate of steepness dropped by 0.04–0.08 when the maximum value of the prior for B_0 was increased to $20SSB_{Max}$. Chilipepper rockfish showed the biggest decrease in mean estimated steepness, with a mean value of 0.52 in scenario S1 compared with the baseline value of 0.6. This was driven by a concentration of posterior density at the bottom end of the range of steepness as the range of the prior for B_0 was increased (see also baseline scenario in Fig. 4). A similar concentration of posterior density was also observed for Goose Island Gully Pa-

Table 6. Posterior mean (standard deviation (SD)) estimates of h and U_{MSY} and mean %SPR_0 at U_{MSY} obtained from the baseline hierarchical models.

	Beverton–Holt			Ricker			
	h	U_{MSY}	%SPR_0	h	U_{MSY}	%SPR_0	M
Posterior predictive mean (SD)	0.67 (0.17)	—	—	0.87 (0.38)	—	—	—
Chilipepper	0.60 (0.18)	0.19 (0.10)	0.41	0.56 (0.22)	0.14 (0.07)	0.49	0.24
Bocaccio	0.50 (0.15)	0.11 (0.05)	0.51	0.45 (0.14)	0.09 (0.04)	0.59	0.20
Widow	0.60 (0.17)	0.17 (0.09)	0.40	0.30 (0.05)	0.04 (0.02)	0.76	0.15
Canary	0.76 (0.05)	0.14 (0.02)	0.32	1.22 (0.14)	0.20 (0.03)	0.25	0.08
Black	0.72 (0.14)	0.30 (0.17)	0.24	0.89 (0.24)	0.24 (0.07)	0.30	0.28
Yellowtail	0.72 (0.12)	0.21 (0.09)	0.30	0.83 (0.17)	0.18 (0.04)	0.34	0.14
POP WC	0.43 (0.03)	0.04 (0.01)	0.58	0.47 (0.04)	0.05 (0.01)	0.55	0.06
POP GIG	0.64 (0.17)	0.08 (0.05)	0.36	0.55 (0.18)	0.05 (0.02)	0.48	0.05
Northern BSAI	0.72 (0.09)	0.06 (0.02)	0.32	0.86 (0.18)	0.06 (0.01)	0.33	0.04
POP BSAI	0.88 (0.06)	0.12 (0.03)	0.21	1.37 (0.24)	0.12 (0.02)	0.22	0.06
Dusky GoA	0.74 (0.10)	0.13 (0.05)	0.29	0.96 (0.24)	0.13 (0.04)	0.30	0.07
Northern GoA	0.70 (0.13)	0.08 (0.04)	0.32	0.84 (0.32)	0.08 (0.03)	0.34	0.06
POP GoA	0.84 (0.07)	0.11 (0.03)	0.24	1.19 (0.21)	0.11 (0.02)	0.24	0.06
Rougheye GoA	0.75 (0.11)	0.06 (0.02)	0.29	0.99 (0.34)	0.06 (0.02)	0.29	0.03

Note: Values of M are also shown for comparison. Posteriors, including the mean of the posterior predictive distribution, were obtained using data from all 14 populations. See Table 2 for population codes.

cific ocean perch under S1. Both populations had very uninformative data sets. Similarly, several of the Alaskan populations with very uninformative data (dusky rockfish, rougheye rockfish, and both populations of northern rockfish) also showed strong sensitivity to the range of the prior used for B_0. These downward shifts in posterior probability estimates for individual populations had the effect of reducing the mean of the posterior predictive distribution from 0.67 to 0.64.

Using the data to select the upper limit of the B_0 prior for individual populations (scenario S2) did curb the tendency of the model to sample from the extreme right-hand tail of the posterior distribution for some populations and resulted in estimates of steepness closer to the baseline estimates, although there was still a slight drop in mean estimated steepness for many populations (Table 7). The mean of the posterior predictive distribution was reduced from 0.67 to 0.66 under this scenario.

Posterior estimates of steepness were almost completely insensitive to the range of values used in the prior for the precision of the recruitment estimates τ (scenario S3; Table 7).

Sensitivity to M

Unsurprisingly, results were very sensitive to the assumptions made about M, although inclusion of age-varying estimates of M for canary, black, and yellowtail rockfishes (scenario S4) had a relatively minor effect on final estimates of steepness. The posterior mean estimate of steepness for canary rockfish was reduced from 0.76 to 0.72 (Table 7). Estimates for black and yellowtail rockfishes changed from 0.72 to 0.74 and 0.71, respectively (Table 7).

Increasing M by 20% (scenario S5) resulted in lower estimates of steepness for all populations (Table 7). Correspondingly, the mean of the posterior predictive distribution was reduced from 0.67 to 0.62. Decreasing M by 20% (scenario S6) had the opposite effect, resulting in higher estimates of steepness for individual populations and an increase in the mean of the posterior predictive distribution from 0.67 to 0.74, with a slight decrease in the SD.

Posterior estimates of U_{MSY} were affected predictably by changes in posterior estimates of steepness, with mean estimates of U_{MSY} increasing with increases in h (Table 7). Similarly, posterior mean estimates of %SPR_0 at U_{MSY} tended to decrease with increases in h and vice versa (Table 8).

Effect of excluding individual data sets

One population (West Coast Pacific ocean perch) had a strong effect on the posterior predictive distribution of steepness (Fig. 9). Box plots indicate that the median of the posterior predictive distribution, when data for West Coast Pacific ocean perch were excluded, was approximately 10% higher than when this population was included. The marginal posterior distribution of h for this population was considerably lower and more precise than for other populations (Fig. 4; Table 6) and had the effect of pulling the posterior predictive distribution towards a lower range of values. The posterior predictive distribution for h was largely insensitive to exclusion of any other population (Fig. 9).

Discussion

Hierarchical Bayesian meta-analysis has been used by a number of authors as a means of overcoming the problem of uninformative data in estimation of productivity parameters (Liermann and Hilborn 1997; Michielsens and McAllister 2004; Su et al. 2004). Hierarchical meta-analysis has the advantage of reducing uncertainty in parameter estimates for data-limited populations by incorporating data from multiple similar populations simultaneously (Gelman et al. 1995).

Data sets for most of the populations considered in this study were relatively uninformative, resulting in imprecise marginal posterior distributions for most populations. The posterior predictive distribution for steepness was similarly

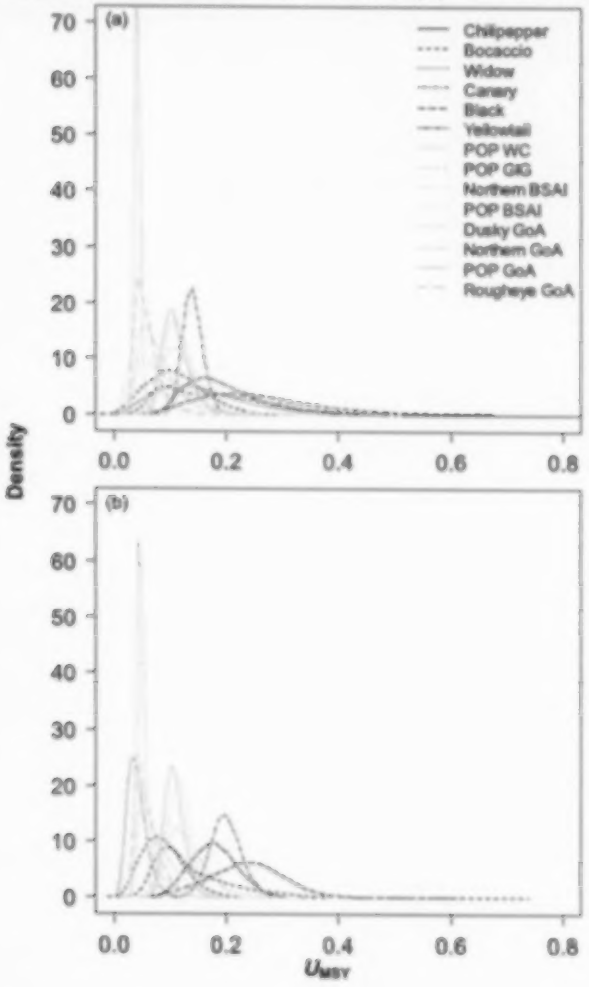


Fig. 5. Marginal posterior probability distributions of U_{MSY}, derived numerically from the posteriors for h in (*a*) Beverton–Holt and (*b*) Ricker hierarchical models. See Table 2 for population codes.

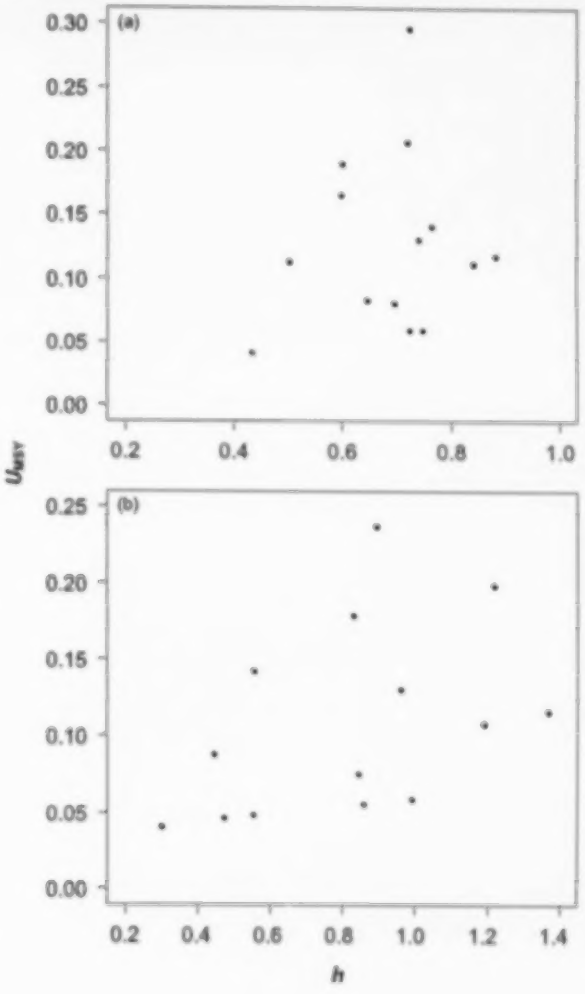


Fig. 6. Relationship between mean of the posterior estimates of h and U_{MSY} from (*a*) Beverton–Holt and (*b*) Ricker hierarchical models.

imprecise with a mean around 0.67 (SD = 0.17) under the assumption of Beverton–Holt recruitment and 0.87 (SD = 0.38) under Ricker recruitment. The mean of the Beverton–Holt posterior predictive distribution for steepness obtained here is consistent with the mode of the posterior predictive distribution reported by Dorn (2002), who reported the highest probability for the bin with midpoint 0.725. To the best of our knowledge, Michielsens and McAllister (2004) were the first to publish a transformation between α and steepness for the Ricker model that accounts for the fact that steepness can take values greater than unity. Dorn (2002) used the transformation given in Myers et al. (2002), which provides the value of steepness for a Beverton–Holt model and thus would never take values greater than unity. We therefore do not directly compare our posterior predictive distributions obtained in the Ricker case with those of Dorn (2002). For individual populations, the marginal Beverton–Holt posterior probability distributions obtained here tended to be higher and more precise than those of Dorn (2002), although this is not really surprising given the addition of several new populations with high predicted steepness and the addition of new data for several populations. Sensitivity analysis suggested that differences might have been partially due to differences in the formulation of priors of B_0. Both Dorn (2002) and Brodziak (2002) reported that data for Pacific rockfishes were not informative enough to distinguish between the Ricker and Beverton–Holt models. We therefore recommend that uncertainty in management advice arising from this structural uncertainty be explicitly acknowledged in future assessments.

Results of our analysis suggest that although there were large differences in posterior estimates of steepness among populations (both within the same hierarchical model and between Beverton–Holt and Ricker models), the range of posterior mean estimates for U_{MSY} among populations was reasonably small.

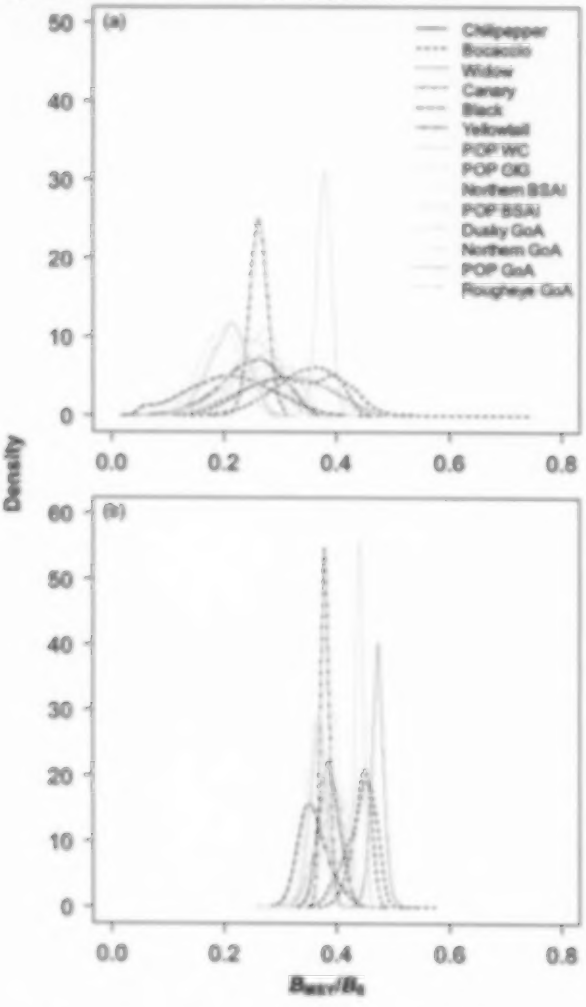


Fig. 7. Marginal posterior probability distributions of B_{MSY}/B_0 under the two different types of recruitment obtained from (*a*) Beverton–Holt and (*b*) Ricker hierarchical models. Biomass refers to spawning stock biomass. See Table 2 for population codes.

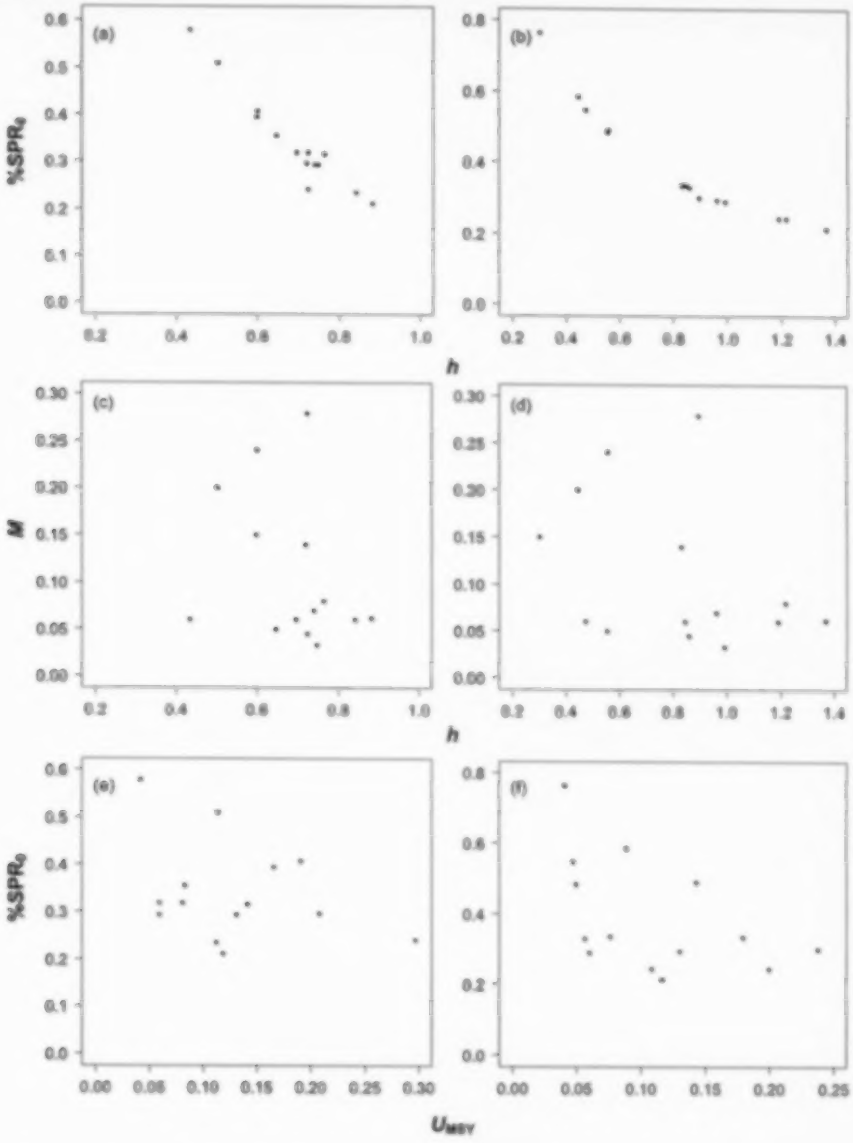
We elected to use the discrete form of the survivorship function and present our results in terms of the annual exploitation rate U rather than instantaneous fishing mortality rate F. In a full stock assessment, it would be more appropriate to use the instantaneous formulation, which does not assume separation of natural and fishing mortality and avoids the use of difference equations that can cause problems if biomass is predicted to be less than the catch at any point during simulation runs. In our analysis, we used the discrete form for convenience, as it is computationally more efficient and allowed us to show results in terms of the proportion of the population that can be sustainably harvested, rather than the less intuitive instantaneous rate. Given the low values of U_{MSY} obtained for these species (for values less than 0.2, F and U are similar in value), this is unlikely to have had a major effect on estimated management parameters. For all populations except black and yellowtail rockfishes, posterior mean estimates of U_{MSY} were less than 0.2 under both assumptions about the stock–recruit relationship and in all except one of the sensitivity analyses we considered (scenario S6). For several populations, notably the West Coast and Goose Island Gully Pacific ocean perch populations, rougheye rockfish, and both populations of northern rockfish, mean U_{MSY} was less than or equal to 0.1 under both stock–recruit assumptions and in all sensitivity analyses. Under the assumption of Ricker recruitment, bocaccio and widow rockfish also had mean estimates of U_{MSY} in this range.

In general, estimates of U_{MSY} obtained from the Beverton–Holt models were higher than those obtained from the Ricker models, although this was not always the case. This carried through to estimates of B_{MSY}/B_0 and $\%SPR_0$ at U_{MSY}, which tended to be higher (i.e., more conservative) under the assumption of Ricker recruitment in most cases. This is consistent with expectations of lower estimates of α from the Ricker model when extrapolating beyond the range of the data for populations with uninformative data (Walters and Martell 2004). It is interesting to note, however, that for the same given value of α, the Ricker model will tend to predict a higher value of U_{MSY} than the Beverton–Holt model due to the different functions describing the relationship between α and U_{MSY} (Forrest 2008), hence the inconsistency in some cases as to which stock–recruit function predicted higher mean U_{MSY}. Therefore, even though α is equivalent in both Ricker and Beverton–Holt stock–recruit functions (Myers et al. 1999), it is very important to consider which function to use, as the different functions will, in most cases, produce different management advice.

Populations with higher estimated steepness did not necessarily have higher estimated U_{MSY}. Although recruitment compensation is a key determinant of U_{MSY}, other factors are also important, notably growth rate (the rate at which maximum weight is attained), natural mortality rate, age at maturity, and age at which fish are first captured. The relationship between h and U_{MSY} is unique to an individual population and is determined by its life history attributes and the selectivity schedule imposed on it (Forrest et al. 2008; Forrest and Walters 2009). From biological and management perspectives, this means that life history, selectivity, and recruitment compensation all play a role in determining U_{MSY} and the most appropriate harvest strategy. In the present study, around half of the populations (notably the Alaskan populations and the Pacific ocean perch populations) had very low estimates of M (≤ 0.07 year^{-1}) and high estimated h. Corresponding values of U_{MSY} were very low, despite very high steepness. These results serve as a reminder that evidence of high steepness per se is insufficient to make statements about sustainable harvest rates, as other factors, particularly natural mortality and the selectivity schedule, must be considered simultaneously.

One of the primary aims of this analysis was to provide a posterior predictive distribution of steepness that could be used as a prior in assessments of populations similar to the ones included in the meta-analysis. The posterior predictive distribution incorporates data from all of the populations in the meta-analysis, and because of the assumption of exchangeability inherent in hierarchical meta-analysis, it is most

Fig. 8. Relationship between (*a*, *b*) mean %SPR_0 at U_{MSY} and mean *h*; (*c*, *d*) mean *h* and *M*; and (*e*, *f*) mean %SPR_0 at U_{MSY} and mean U_{MSY} from the Beverton–Holt (left) and Ricker (right) models for the 14 Pacific rockfish populations. All means are means of the estimated posterior distributions.



appropriate to use the posterior predictive distribution as a prior for steepness in assessment of a new population rather than the marginal posterior distribution for any individual population, even if the new population is the same species as one already in the meta-analysis. On a note of caution, the posterior predictive distribution cannot be used as a prior for any population already included in the meta-analysis, as this is in violation of Bayesian principles (Minte Vera et al. 2005). In such cases, the meta-analysis should be re-run without the population of interest and the resulting posterior predictive distribution used as a prior in the new analysis.

It is possible that inclusion of the West Coast Pacific ocean perch population in the analysis resulted in violation of the assumption of exchangeability among data sets. Exclusion of the data set for West Coast Pacific ocean perch had a large effect on both the location and precision of the posterior predictive distribution for steepness. None of the other data sets had a noticeable effect when excluded. It is therefore possible that our posterior estimates of steepness are biased downwards. Resulting estimates of U_{MSY} would then also be biased downwards. It might be argued that it would have been more appropriate to remove the data set for this population from the analysis and report results without it. The West Coast Pacific ocean perch population lives

Table 7. Posterior mean (and standard deviation (SD)) estimates of h and mean U_{MSY} obtained from the Beverton–Holt hierarchical model in sensitivity analyses S1–S6.

	S1	x	S2	S3	S4	S5	S6
h							
Posterior predictive mean (SD)	0.64 (0.18)		0.66 (0.17)	0.67 (0.17)	0.68 (0.16)	0.62 (0.18)	0.74 (0.14)
Chilipepper	0.52 (0.18)	5	0.61 (0.17)	0.60 (0.018)	0.62 (0.17)	0.53 (0.20)	0.70 (0.14)
Bocaccio	0.45 (0.15)	10	0.48 (0.15)	0.50 (0.15)	0.53 (0.15)	0.45 (0.15)	0.61 (0.14)
Widow	0.56 (0.17)	10	0.58 (0.17)	0.60 (0.17)	0.62 (0.17)	0.56 (0.18)	0.67 (0.15)
Canary	0.76 (0.05)	10	0.76 (0.05)	0.76 (0.05)	0.72 (0.05)	0.71 (0.05)	0.82 (0.04)
Black	0.69 (0.16)	10	0.71 (0.14)	0.72 (0.14)	0.74 (0.13)	0.66 (0.17)	0.79 (0.11)
Yellowtail	0.70 (0.12)	10	0.71 (0.12)	0.72 (0.12)	0.71 (0.12)	0.66 (0.14)	0.79 (0.09)
POP WC	0.42 (0.03)	20	0.42 (0.03)	0.43 (0.03)	0.51 (0.03)	0.36 (0.03)	0.53 (0.03)
POP GIG	0.58 (0.18)	10	0.64 (0.17)	0.65 (0.17)	0.66 (0.16)	0.59 (0.19)	0.73 (0.14)
Northern BSAI	0.65 (0.09)	20	0.65 (0.09)	0.72 (0.09)	0.72 (0.09)	0.63 (0.11)	0.82 (0.07)
POP BSAI	0.88 (0.06)	10	0.88 (0.06)	0.88 (0.06)	0.88 (0.06)	0.85 (0.08)	0.91 (0.04)
Dusky GoA	0.68 (0.10)	20	0.69 (0.10)	0.74 (0.10)	0.74 (0.10)	0.66 (0.12)	0.82 (0.08)
Northern GoA	0.63 (0.14)	15	0.66 (0.14)	0.69 (0.13)	0.70 (0.13)	0.62 (0.16)	0.78 (0.09)
POP GoA	0.83 (0.07)	10	0.84 (0.07)	0.84 (0.07)	0.84 (0.07)	0.80 (0.08)	0.88 (0.05)
Rougheye GoA	0.69 (0.13)	15	0.72 (0.12)	0.75 (0.11)	0.75 (0.11)	0.68 (0.14)	0.82 (0.08)
U_{MSY}							
Chilipepper	0.15 (0.09)	5	0.19 (0.10)	0.19 (0.10)	0.20 (0.10)	0.15 (0.11)	0.24 (0.09)
Bocaccio	0.09 (0.05)	10	0.11 (0.05)	0.11 (0.05)	0.12 (0.05)	0.09 (0.05)	0.15 (0.05)
Widow	0.15 (0.09)	10	0.16 (0.09)	0.17 (0.10)	0.17 (0.09)	0.15 (0.10)	0.20 (0.09)
Canary	0.14 (0.02)	10	0.14 (0.02)	0.14 (0.02)	0.19 (0.02)	0.12 (0.02)	0.17 (0.02)
Black	0.27 (0.17)	10	0.29 (0.16)	0.29 (0.17)	0.28 (0.10)	0.25 (0.16)	0.35 (0.17)
Yellowtail	0.19 (0.09)	10	0.20 (0.09)	0.21 (0.09)	0.24 (0.09)	0.18 (0.09)	0.25 (0.09)
POP WC	0.04 (0.01)	20	0.04 (0.01)	0.04 (0.01)	0.05 (0.01)	0.03 (0.01)	0.06 (0.01)
POP GIG	0.07 (0.05)	10	0.08 (0.05)	0.08 (0.05)	0.09 (0.05)	0.07 (0.05)	0.10 (0.05)
Northern BSAI	0.05 (0.01)	20	0.05 (0.01)	0.06 (0.02)	0.06 (0.02)	0.05 (0.02)	0.08 (0.02)
POP BSAI	0.12 (0.03)	10	0.12 (0.03)	0.12 (0.03)	0.12 (0.02)	0.11 (0.03)	0.13 (0.02)
Dusky GoA	0.11 (0.04)	20	0.11 (0.04)	0.13 (0.05)	0.13 (0.05)	0.11 (0.05)	0.17 (0.05)
Northern GoA	0.07 (0.04)	15	0.07 (0.03)	0.08 (0.04)	0.08 (0.03)	0.07 (0.04)	0.10 (0.03)
POP GoA	0.11 (0.03)	10	0.11 (0.03)	0.11 (0.03)	0.11 (0.02)	0.10 (0.03)	0.12 (0.02)
Rougheye GoA	0.05 (0.02)	15	0.05 (0.02)	0.06 (0.02)	0.06 (0.02)	0.05 (0.02)	0.07 (0.02)

Note: Key to scenarios: S1, upper limit of B_0 prior = $20\,SSB_{max}$; S2, upper limit of B_0 prior = $x\,SSB_{max}$; S3, $\tau \sim \Gamma(0.0001, 0.0001)$; S4, age schedules for M used for canary, black, and yellowtail rockfishes (see Fig. 1); S5, M increased by 20% for all populations; S6, M decreased by 20% for all populations. See Table 2 for population codes.

at the southernmost extreme of the range of this species and so may be atypical. Like other Pacific ocean perch populations, it was severely exploited during the 1960s and 1970s. Unlike populations in the centre of the range (British Columbia and southern Alaska), however, the West Coast population has been slow to recover and may be more influenced by environmental conditions than its more resilient northern counterparts (Dorn 2002). It may not, therefore, share recruitment characteristics with other populations of the same species. However, an alternative interpretation of the results from West Coast Pacific ocean perch is that it represents a legitimate lower range of values for steepness that should be included in the prior for new populations. Without it, the posterior predictive distribution may be overly precise, as the small sample size of the present analysis (14 populations) may have left out extremes. If it had been possible to sample many more rockfish populations, there could have been a more complete distribution of posteriors obtained for steepness, including both high and low values. In so far as Bayesian analysis aims to represent uncertainty in as true a manner as possible, there would be no strong reason to reject West Coast Pacific ocean perch from the analysis. Furthermore, with the exception of canary rockfish, the data for West Coast Pacific ocean perch was the most informative of all the data sets, because it contained many observations at low stock size. The high precision of the posterior for this population was one of the reasons for its strong influence on the results. The question is then whether an informative data set can defensively be rejected from the analysis because it exerts a stronger influence on results than any other data set.

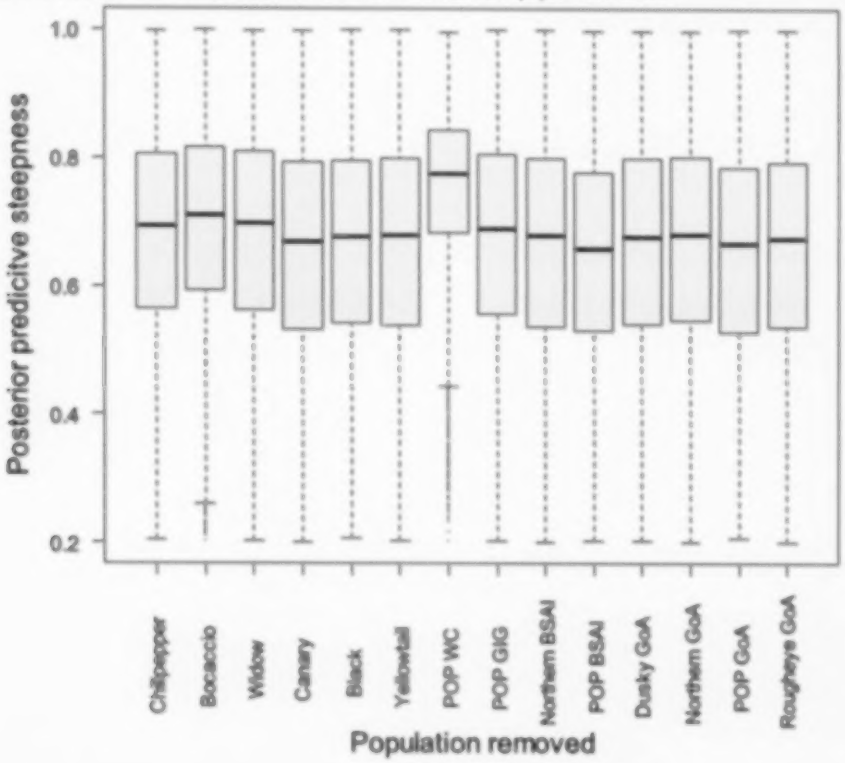
Hierarchical Bayesian meta-analysis rests on the assumption that the different populations in the analysis are exchangeable with respect to the key estimated parameter, i.e., knowing the identity and the associated properties of a given population (e.g., M or vulnerability at age) tells us nothing about the possible value of the parameter for that population (Gelman et al. 1995). Prévost et al. (2003) suggested that the best approach for transferring information from data-rich to data-sparse populations would be to use a meta-analysis that includes one or more covariates (e.g., latitude, natural mortality rate, species) to help predict the parameter of interest

Table 8. Posterior mean estimates of %SPR_0 (calculated using posterior mean estimates of U_{MSY}) obtained from the Beverton–Holt hierarchical model in sensitivity analyses S1–S6.

	S1	x	S2	S3	S4	S5	S6
Chilipepper	0.48	5	0.40	0.41	0.40	0.47	0.34
Bocaccio	0.57	10	0.53	0.51	0.48	0.57	0.42
Widow	0.43	10	0.41	0.40	0.38	0.42	0.34
Canary	0.32	10	0.32	0.32	0.38	0.35	0.28
Black	0.27	10	0.25	0.24	0.29	0.29	0.20
Yellowtail	0.31	10	0.30	0.30	0.32	0.34	0.25
POP WC	0.59	20	0.59	0.58	0.51	0.67	0.49
POP GIG	0.40	10	0.36	0.36	0.35	0.40	0.31
Northern BSAI	0.38	20	0.38	0.32	0.32	0.38	0.25
POP BSAI	0.21	10	0.21	0.21	0.22	0.23	0.19
Dusky GoA	0.34	20	0.33	0.29	0.30	0.35	0.24
Northern GoA	0.37	15	0.35	0.32	0.32	0.37	0.26
POP GoA	0.24	10	0.24	0.24	0.24	0.26	0.21
Rougheye GoA	0.33	15	0.31	0.29	0.30	0.34	0.25

Note: Key to scenarios: S1, upper limit of R_0 prior = $20\,SSB_{min}$; S2, upper limit of R_0 prior = $x\,SSB_{min}$; S3, $\tau \sim \Gamma(0.0001, 0.0001)$; S4, age schedules for M used for canary, black, and yellowtail rockfishes (see Fig. 1); S5, M increased by 20% for all populations; S6, M decreased by 20% for all populations. See Table 2 for population codes.

Fig. 9. Median and quantiles of posterior predictive distribution for steepness from the hierarchical Beverton–Holt model obtained by systematically removing one population at a time. Median estimates are shown as a black bar inside the box showing the interquartile range (IQR). Whiskers represent 1.5 IQR, with outliers shown as dots. See Table 2 for population codes.



(see also Helser and Lai 2004; Helser et al. 2007). We did not take this approach in the current analysis but recognise that it could be a useful extension of this work, given that there may be species or latitudinal effects influencing recruitment parameters and especially given the relationship between natural mortality and steepness.

In a recent study, a hierarchical model was used to improve accuracy in estimates of selectivity parameters in a

set of 21 Canadian lakes (Askey et al. 2007). The hierarchical model replaced highly unrealistic vulnerability and selectivity parameter estimates from an individual-based approach that may have resulted from sparse and imprecise data with more realistic estimates. In their analysis, there was one lake for which it appeared that a markedly better fit to the data was achieved with the individual model than with the hierarchical model, suggesting that the hierarchical analysis may have produced a biased result for this lake. However, the individual model estimated for this lake what the authors judged to be an unrealistically high probability of removal of large fish, whereas the hierarchical model estimate was far more credible. Given the ubiquity of the problem of poor data for parameter estimation, the advantages of hierarchical meta-analysis may outweigh the disadvantages, as long as reasonable care is taken in the choice of data sets to include and appropriate caution is taken with use of the results.

Mean values of $\%SPR_0$ at U_{MSY} were, in general, lower than those reported by Dorn (2002), although they spanned a similar range of values. The addition of the Alaskan populations in this analysis most likely explains the lower estimates of $\%SPR_0$ obtained here compared with those of Dorn (2002). Most of the new populations had low M and high corresponding posterior estimates of steepness, which in turn pulled all of the estimates of steepness upwards in the hierarchical framework. This in turn resulted in a general trend of lower $\%SPR_0$ at U_{MSY}, although it should be remembered that although we have provided mean point estimates of $\%SPR_0$ at U_{MSY}, our results have the same degree of uncertainty as our posterior estimates of U_{MSY}.

The fishery management plan for rockfishes in Alaska stipulates that when F_{MSY} cannot be calculated, a harvest rate corresponding to $F_{40\%}$ should be adopted, whereas on the US West Coast, an F_{MSY} proxy of $F_{50\%}$ is used. Clark (2002) showed that a much more conservative target fishing mortality rate of $F_{50\%}$ was robust to a wide range of uncertainty about productivity of populations (but see Hilborn et al. 2002). The present results suggest that these reference points should be adequate for most of the populations under consideration here, largely because of the low estimates of $\%SPR_0$ at U_{MSY} obtained for the Alaskan populations. Our results suggests that the trade-off between M and steepness results in a certain degree of stability in $\%SPR_0$ at U_{MSY} across populations with different natural mortality rates and steepness, despite the very strong negative relationship between steepness and $\%SPR_0$ at U_{MSY}. Williams (2002) also showed a negative relationship between steepness and $\%SPR_0$ at U_{MSY}, which was robust to age at first harvest, although his calculations were done with fixed life history parameters and his statement that a population with higher steepness can sustain higher harvest rates is only true if all other parameters are held constant. We encourage further investigation of such relationships among different fish populations.

We caution that $\%SPR_0$ should not be interpreted too simplistically. To illustrate, the populations with the lowest natural mortality rates and lowest estimates of U_{MSY} (Pacific ocean perch populations and Alaskan rockfish populations) were estimated here to have the lowest $\%SPR_0$ at U_{MSY}, whereas the populations with the highest natural mortality rates and higher estimates of U_{MSY} (chilipepper, widow, and black rockfishes and bocaccio) had the highest estimated $\%SPR_0$. At first, this appears counterintuitive, as populations with lower natural mortality rates would be expected to need to be maintained at higher population biomass to avoid overfishing. However, populations with low natural mortality rate may also be expected to exhibit a greater degree of recruitment compensation (Dorn 2002; Winemiller 2005; Goodwin et al. 2006), resulting in higher recruitment rates at low population size. The trade-off between M and steepness implies that populations with high steepness may have lower U_{MSY} than would be expected from knowledge of steepness alone. In addition, even though $\%SPR_0$ at U_{MSY} may be low for such populations (due to high steepness), lower M and U_{MSY} means that even if productivity is maximized when spawning stock biomass is low, the proportion of the population that can be sustainably harvested will still be low. It is therefore a mistake to assume that low $\%SPR_0$ at U_{MSY} for a population is evidence that the population can be fished harder. For slow-growing populations such as some rockfishes, even low harvest rates can drive the population to very low levels.

SPR harvest rate policies can be implemented either through control of absolute catch (which relies on reliable estimates of catch and population biomass) or through direct control of harvest rate (where the harvest rate is measured, for example, through a tagging program). Hilborn et al. (2002) and Clark (2002) caution against the use of management strategies that rely on estimates of current and unfished biomass. Fixed harvest rate policies may be more robust to uncertainty in population size and are also robust to environmental fluctuation (Walters and Parma 1996; Martell and Walters 2002) but, obviously, cannot be implemented in the absence of estimates of U_{MSY}. Priors for U_{MSY} based on results of analyses such as ours and that of Forrest and Walters (2009) or from simpler proxies (e.g., Gulland 1971; Patterson 1992; Beddington and Kirkwood 2005) may be suitable for use in this management approach. Hilborn et al. (2002) proposed an alternative feedback mechanism based on catch for managing fish stocks in the absence of estimates of U_{MSY} and reliable estimates of population size. Alternatively, efforts to adjust selectivity schedules so that all individuals have had the opportunity to spawn before harvesting can greatly reduce the probability of growth and recruitment overfishing (Myers and Mertz 1998; Froese et al. 2008).

Limitations

Our analysis assumed that life history parameters and selectivity schedules were stationary and perfectly known and that the estimates of stock and recruitment obtained from previous assessment models were representative of the true underlying values. We caution that our estimates of U_{MSY} were strongly determined by the estimates of natural mortality and age-specific selectivity that were used in the models. This is particularly important because the terminal age reported for most populations was much lower than the maximum reported age (i.e., many species of rockfishes live for more than 50 years; Love et al. 2002). Although a plus group was used to account for older age classes, this included the implicit assumption that selectivity on older age

classes was the same as for the reported terminal age. This may not be a serious issue for our analysis, as age classes older than those observed in the fishery tend to become rare after years of fishing. We also assumed stationary growth parameters (i.e., growth not influenced by population size or by environmental conditions). This is a common assumption in fisheries models, and although we recognise that wrong assumptions about growth can affect model results (Sainsbury 1980), it was beyond the scope of this study and the available data to fully account for nonstationary growth effects. Due to the necessary assumptions about constant M and growth and selectivity parameters, the estimated posterior distributions might be considered overly precise.

The natural mortality rate M is often assigned a point value in stock assessment, and in our use of the data, we did not distinguish between stock assessments that estimated M and those that made an assumption about its value. We recognise that our assumptions about M are a limitation of the analysis but suggest that admitting uncertainty in M and attempting to estimate it alongside other parameters would have resulted in overparameterised models due to lack of contrast in the data. We therefore tested sensitivity of the model outputs to the assumed values of M by simulating underestimation and overestimation of M. Results indicated that increasing M led to decreases in the mean estimates of steepness and a corresponding increase in the mean $\%SPR_0$ at U_{MSY}. This implies that if the true value were 20% higher than the assumed value, steepness would be overestimated and $\%SPR_0$ at U_{MSY} would be underestimated. This may appear counterintuitive, as one would expect that underestimating M would lead to a more cautious estimate of $\%SPR_0$ at U_{MSY}. However, lower assumed values of M led to higher estimates of steepness, which we have shown to be very strongly negatively correlated with $\%SPR_0$ at U_{MSY}. Alternatively, low values of M result in higher steepness and correspondingly lower values of $\%SPR_0$ at U_{MSY}, implying that if the assumed value of M is higher than the true value, steepness will be underestimated and $\%SPR_0$ at U_{MSY} will be overestimated. These seemingly counterintuitive results underline the importance of sensitivity analysis for important parameters that are assumed fixed in stock assessments.

Our results were influenced by the assumption that egg production is directly proportional to female body weight. This may be a poor assumption for some rockfish populations, as there is evidence that larger, older females may contribute disproportionately to the production of larvae than females in younger age classes. This is understood to be achieved through production of more viable larvae and more larvae per unit of spawning biomass by older females (Berkeley et al. 2004; Sogard et al. 2008). We did not include such correction factors in our analysis because data were not available for most species (although some data were available for bocaccio, chilipepper, and widow rockfishes). All other things being equal, the effect of ignoring maternal effects on recruitment could be overestimation of U_{MSY} if reproductive success of younger females is overestimated. However, the magnitude of the bias would depend on the natural mortality schedule (proportion of females in older age classes) and, importantly, on the selectivity schedule. Under dome-shaped selectivity, where a proportion of older females is invulnerable to fishing gear, there may be little effect of incorrect assumptions about maternal effects on estimates of U_{MSY} and other reference points. We suggest that future analyses incorporate information about relative fecundity at age if it is available. Sensitivity analyses can be used to determine the importance of this effect.

It is common for stock–recruit analysis to use results of previous assessments as data. Dorn (2002) discussed this issue with respect to Pacific rockfishes, assessments of which are generally data-poor. He cautioned that spurious trends in population size resulting from imprecise survey indices could bias results. Time-series bias (Hilborn and Walters 1992) caused by nonindependence of population size and recruitment in time series of stock–recruit data is also a possible problem, although Dorn (2002) notes that most of the rockfish populations considered have been fished from a pristine state without reversal in the biomass trend (i.e., a one-way trip), which would reduce the possibility of this type of bias. Although there may be concerns with using model outputs as data, due care was taken in this analysis not to use data generated by models with strong influence of a stock–recruit function (which could lead to overly precise posteriors in the meta-analysis) and no data sets had been generated using a prior for steepness. Direct observations of stock size and recruitment are rare and usually extremely noisy. Use of model outputs in meta-analysis such as this can provide useful insights and can be worthwhile, despite the limitations of the data.

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Appendix A

Figures A1 and A2 follow.

Fig. A1. Scatterplots showing posterior samples of h vs. B_0 for the 14 rockfish populations from the hierarchical Beverton–Holt model, with the upper limit of the priors for B_0 set to $10\,SSB_{Max}$. Units of biomass vary among populations. See Table 2 for population codes.

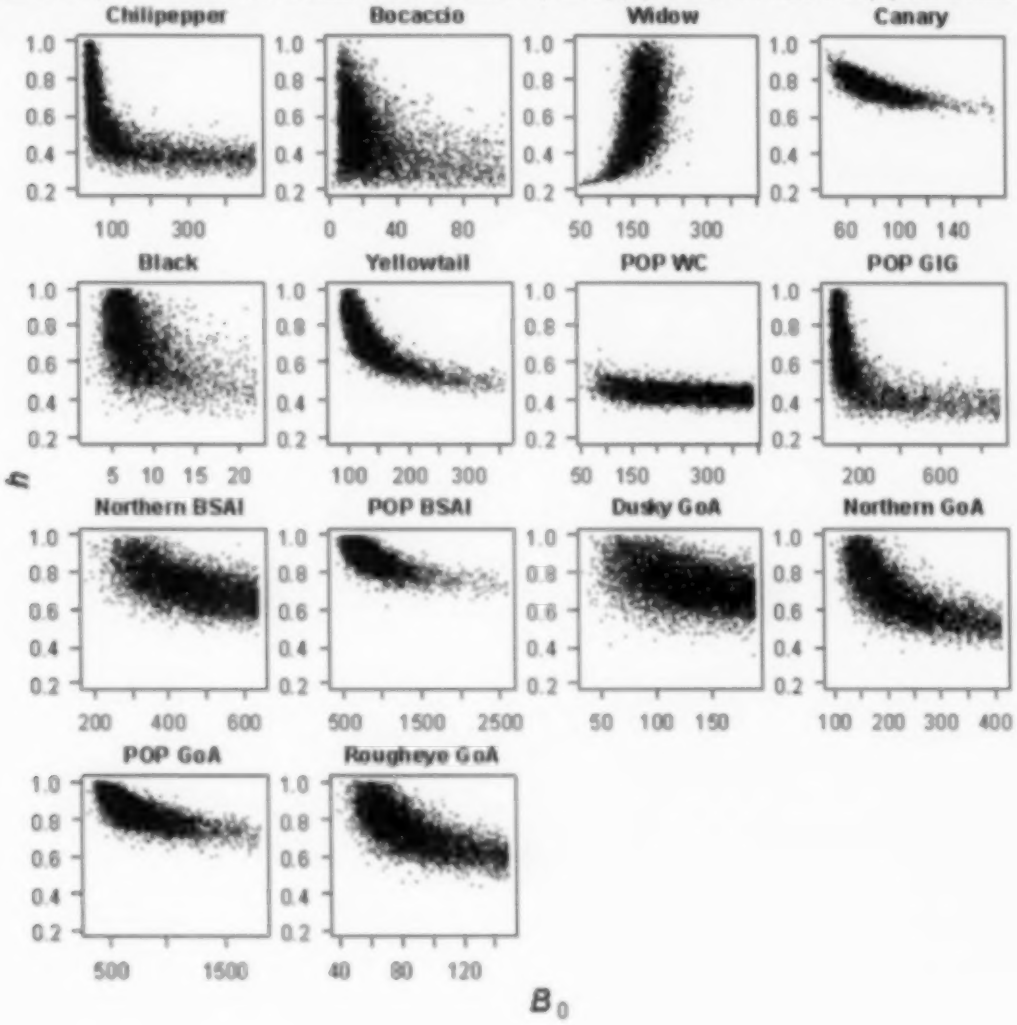
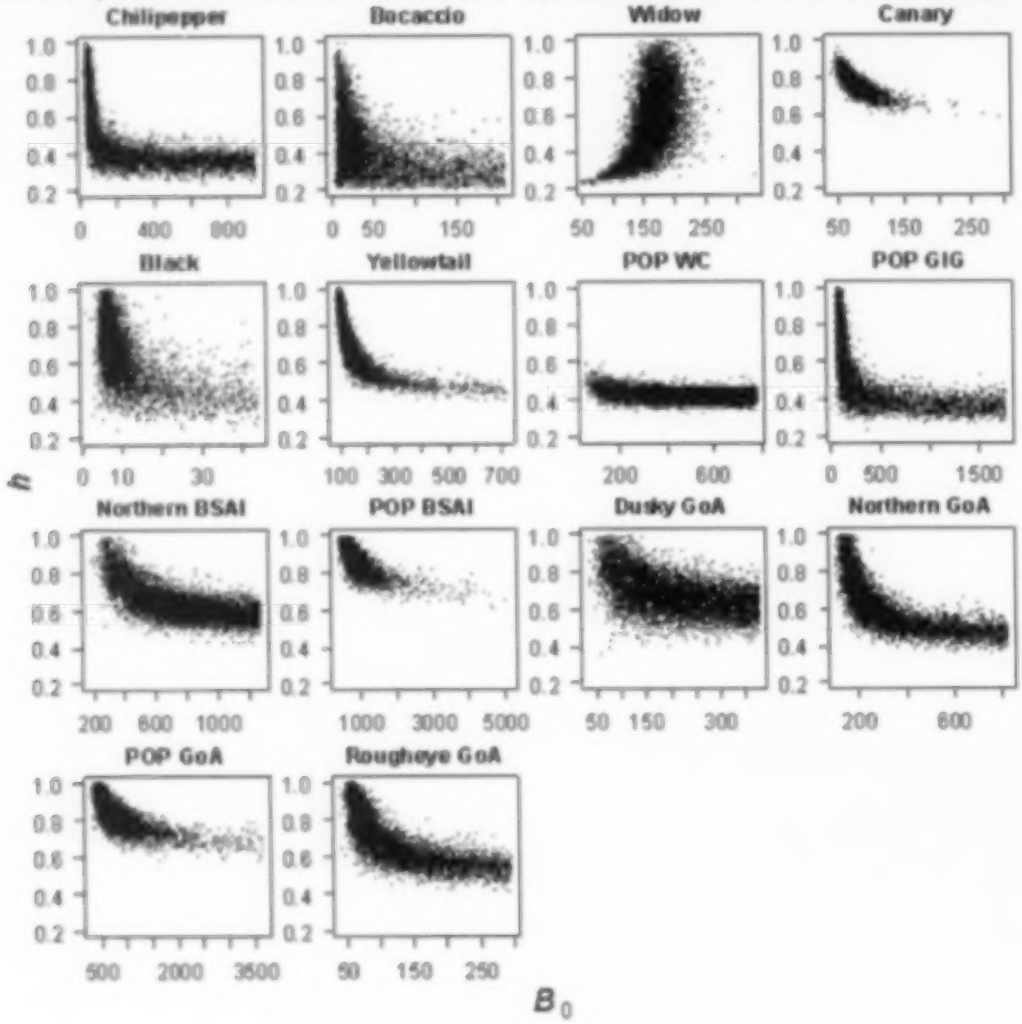


Fig. A2. Scatterplots showing posterior samples of h vs. B_0 for the 14 rockfish populations from the hierarchical Beverton–Holt model, with the upper limit of the priors for B_0 set to $20SSB_{Max}$. Units of biomass vary among populations. See Table 2 for population codes.



Direct and indirect effects of fish on pelagic nitrogen and phosphorus availability in oligotrophic Arctic Alaskan lakes

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Abstract: The importance of fish nutrient recycling for lake primary production increases with lake productivity. However, fish in low-productivity lakes may have substantial indirect effects on nutrient recycling from lower trophic levels. We measured nutrient excretion rates from fish and zooplankton in oligotrophic Arctic lakes and investigated direct and indirect fish effects on consumer nutrient recycling. Fish nutrient excretion rates were small relative to phytoplankton nutrient demand. Zooplankton excretion, however, supplied 19%–130% and 37%–200% of phytoplankton nitrogen and phosphorus demand, respectively. Fish had a significant effect on zooplankton biomass; in lakes with fish, this was approximately 80% lower than in lakes without fish. The difference in zooplankton biomass was due primarily to a decrease in zooplankton density; no significant difference in average zooplankton size was observed between fish and fishless lakes. Fish also impacted zooplankton community composition; communities in lakes with fish were dominated by copepods compared with cladoceran dominance in lakes without fish. Because of lower zooplankton biomass, lakes with fish showed lower rates of zooplankton nitrogen and phosphorus excretion relative to lakes without fish. Thus, our results support the hypothesis that fish have major indirect effects on lake nutrient cycles, even when direct excretion from fish is minimal.

Résumé : L'importance du recyclage des nutriments provenant des poissons pour la productivité primaire lacustre augmente en fonction de la productivité du lac. Cependant, les poissons dans les lacs de faible productivité peuvent avoir des effets significatifs indirects sur le recyclage des nutriments à partir des niveaux trophiques inférieurs. Nous avons mesuré les taux d'excrétion de nutriments des poissons et du zooplancton dans des lacs arctiques oligotrophes et étudié les effets directs et indirects des poissons sur le recyclage des nutriments des consommateurs. Les taux d'excrétion des nutriments par les poissons sont faibles par rapport aux besoins en nutriments du phytoplancton. L'excrétion du zooplancton, cependant, fournit respectivement 19–130 % et 37–200 % de la demande du phytoplancton en azote et en phosphore. Les poissons ont un effet significatif sur la biomasse du zooplancton qui est environ 80 % plus basse dans les lacs avec poissons que dans les lacs sans poissons. La différence de biomasse du zooplancton est due principalement à une diminution de la densité du zooplancton, car on n'observe aucune différence significative de la taille moyenne du zooplancton entre les lacs avec et sans poissons. Les poissons affectent la composition de la communauté de zooplancton : les communautés des lacs avec poissons sont dominées par les copépodes, alors que les cladocères prédominent dans les lacs sans poissons. À cause de leur biomasse réduite de zooplancton, les lacs avec poissons ont des taux plus bas d'excrétion d'azote et de phosphore par le zooplancton que les lacs sans poissons. Nos résultats appuient ainsi l'hypothèse selon laquelle les poissons ont des effets indirects significatifs sur le recyclage des nutriments dans les lacs, même lorsque l'excrétion directe des poissons est très faible.

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Introduction

In many lakes autogenic recycling of nutrients is the dominant source of inorganic nitrogen (N) and phosphorus (P) for algal and bacterial production (Hudson et al. 1999; Vanni et al. 2006; Kamarainen et al. 2009). Rates of nutrient regeneration and uptake are affected by abiotic and biotic drivers, such as ultraviolet radiation (Allen and Smith 2002), resuspension from lake sediments (Fortino et al. 2009), temperature (Zimmer et al. 2006), and trophic structure (Vanni et al. 1997). In addition, control of nutrient recycling by fish has been investigated for decades (Brabrand et al. 1990). However, whether nutrient recycling by fish is large relative to ecosystem demand (Persson 1997; McIntyre

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et al. 2008) or small compared with other nutrient sources (Hudson et al. 1999; Sarnelle and Knapp 2005) varies considerably among lakes. In addition, the indirect effects that fish have on lake nutrient cycles (Vanni and Layne 1997) are still unclear even though the topic has received considerable attention (Vanni 2002).

Generally speaking, fish have the greatest direct impact on phytoplankton nutrient availability when fish assemblages are detritivorous (Torres and Vanni 2007; Sereda et al. 2008), water temperatures are warm (Schindler and Eby 1997), and when fish community biomass (Vanni et al. 2006) and density (Verant et al. 2007) are relatively high. By comparison, fish may have important indirect effects on zooplankton nutrient recycling rates, even when conditions are such that direct excretion of N and P by fish is negligible (Schindler et al. 1993; Attayde and Hansson 2001). Excretion rates are inversely related to organism body size (Hall et al. 2007), and fish that selectively eliminate large-bodied zooplankton (sensu Brooks and Dodson 1965) can increase nutrient recycling rates from zooplankton communities (Bartell 1981). Conversely, predation by fish can reduce total zooplankton biomass (Parker and Schindler 2006) and may result in lower rates of nutrient excretion from zooplankton communities. As such, fish may indirectly have a negative impact on phytoplankton production, if a reduction in total zooplankton biomass outweighs the positive effects from higher zooplankton mass-specific excretion rates and lower zooplankton grazing pressure (Ramcharan et al. 1996).

Understanding fish controls over lake nutrient cycling and phytoplankton productivity may be especially important in Arctic lakes (Nakashima and Leggett 1980). Currently, our understanding of Arctic lake nutrient budgets focuses on the surrounding watershed as an external source of N and P. Nutrients are delivered to lakes during high runoff events such as spring snowmelt (Whalen and Cornwell 1985) or summer storm events (MacIntyre et al. 2006). However, runoff from winter snow pack as well as the frequency and intensity of storm events can vary considerably from season to season. When runoff is low, terrestrial plants can retain close to 100% of mineralized nutrients (Shaver et al. 1992). As a result, nutrient loading to Arctic lakes can fluctuate within and between summer growing seasons, and internal sources of N and P may become increasingly important for phytoplankton production as external sources attenuate during periods of low runoff.

When present, fish exert a strong top-down control over lake food webs in Arctic Alaska (Hershey et al. 1999) and can affect the abundance and community composition of zooplankton (O'Brien et al. 2004) and benthic invertebrates (Merrick et al. 1991; Goyke and Hershey 1992), as well as habitat availability for juvenile fish (McDonald et al. 1992; Keyse et al. 2007). However, the food resources for pelagic fish species, which are typically piscivorous at lower latitudes, are poor in Arctic Alaskan lakes because of ultra-oligotrophic conditions and an absence of species of pelagic prey fish. As a result, the abundance of fish in these lakes is low, and individuals are relatively small and near physiological limits for survival (McDonald et al. 1996). Therefore, in Arctic Alaskan lakes it is likely that the potential for fish to influence phytoplankton nutrient availability is greater through indirect controls over zooplankton communities rather than from direct excretion of N and P from fish populations.

The importance of internal nutrient recycling as a nutrient source for phytoplankton production has not been quantified in Arctic Alaskan lakes, nor has the hypothesis that fish can affect bottom-up nutrient availability by the top-down control of lower trophic levels been tested. The presence and composition of fish communities can be accurately predicted in these lakes using landscape variables (Hershey et al. 2006). Therefore, clarifying the role fish play in lake nutrient cycles can be important for understanding phytoplankton nutrient availability in lakes across the landscape.

We hypothesized that direct excretion of N and P by fish would be a relatively unimportant component of pelagic nutrient budgets in Arctic Alaskan lakes because of cold water temperatures and small fish population sizes. However, because fish predation has a considerable top-down influence on zooplankton communities in lakes in this region (O'Brien et al. 2004), we also hypothesized that fish would have significant indirect effects on zooplankton nutrient recycling rates. Finally, because watershed nutrient loading to Arctic Alaskan lakes can be minimal during the relatively dry summer growing season, we predicted that N and P recycled by zooplankton would constitute a relatively large proportion of the nutrients required to sustain phytoplankton growth.

Materials and methods

Site description

Our research was conducted in lakes near Toolik Field Station (68°37.796′N, 149°35.834′W), site of the Arctic Long Term Ecological Research project, located in the northern piedmont region of the Brooks Mountain Range, Arctic Alaska, USA. This area is underlain by continuous permafrost, and regional lakes are typically shallow (maximum depth between 3 and 30 m) moraine dammed or kettle basins. There is a short, approximately 3-month, ice-free season from late June to early October, and daylight is 24 h though most of the summer ice-free season (Toolik Naturalist Journal Annual Summaries, University of Alaska Fairbanks, Institute of Arctic Biology, Toolik Field Station; http://toolik.alaska.edu/edc/journal/annual.php, December 2010). Lakes of intermediate (2.5–4.5 m) or deep (≥4.5 m) maximum depths are usually dimictic and thermally stratify shortly after ice-out (Whalen et al. 2008). However, shallow (maximum depth ≤ 2.5 m) and some intermediate depth lakes can be polymictic and vertically mix on a diel basis or during high wind and storm events (MacIntyre et al. 2006; Whalen et al. 2008; Fortino et al. 2009). In addition, lakes in this region are characteristically ultra-oligotrophic with chlorophyll *a* concentrations typically below 5 $\mu g \cdot L^{-1}$ and concentrations of inorganic N and P near the limits of detection (average 0.22 $\mu mol \cdot L^{-1}$ NO_3, 0.75 $\mu mol \cdot L^{-1}$ NH_4, and 0.8 $\mu mol \cdot L^{-1}$ PO_4) (O'Brien et al. 2004). Water clarity is atypically low for ultra-oligotrophic lakes because of high concentrations of dissolved organic matter, and Secchi depths rarely exceed 6 m (O'Brien et al. 2004)

Fish species richness is low in these lakes because of the geographical barrier to colonization in the south from the Brooks Mountain Range, which runs east to west and iso-

lates the lakes from watersheds with greater species richness draining into the Gulf of Alaska (Hershey et al. 1999). In a survey of 96 lakes, O'Brien et al. (2004) found that 74% contained fish communities, and Arctic grayling (*Thymallus Arcticus*) were the most common pelagic fish species present (47 lakes) followed by lake trout (*Salvelinus namaycush*) (30 lakes), Arctic char (*Salvelinus alpinus*) (20 lakes), and round whitefish (*Prosopium cylindraceum*) (6 lakes). Pelagic mesozooplankton species richness is also relatively low, with only seven common species identified in these lakes (O'Brien et al. 2004). Of these seven species, there are three copepod taxa, two small-bodied species *Cyclops scutifer* and *Diaptomus pribilofensis*, and one large-bodied invertebrate predator *Heterocope septentrionalis* (O'Brien et al. 1979*a*). Common cladoceran taxa in lake zooplankton communities consist of two large-bodied species (*Daphnia middendorffiana* and *Holopedium gibberum*) and two small-bodied species (*Daphnia longiremis* and *Bosmina longirostris*) (O'Brien et al. 1979*a*).

We used a series of six lakes that were in close proximity to Toolik Field Station to estimate the N and P recycled by fish and zooplankton consumer groups at the lake ecosystem level and to determine the proportion of phytoplankton nutrient demand supplied by recycled nutrients in fish and fishless systems. We chose these lakes based on their accessibility from Toolik Field Station and the availability of extant primary production and multiple-year fish mark–recapture data. Three of the study lakes contained fish populations (NE-12, GTH 86, Fog-2), and three of the lakes were fishless (GTH 114, GTH 99, E-4) (Table 1).

We calculated the contribution of consumer nutrient recycling to Arctic lake nutrient budgets by (*i*) empirically measuring excretion rates from fish and zooplankton; (*ii*) estimating the biomass of the major fish and zooplankton consumer groups in Arctic lake food webs; (*iii*) using the measured excretion rates and biomass estimates to calculate ecosystem N and P recycling rates from each of these consumer groups; and (*iv*) comparing rates of N and P recycled from consumer groups to rates of phytoplankton nutrient uptake.

Fish excretion rates

During the summers of 2005, 2007, and 2008, we empirically measured in situ excretion rates of NH_4^+-N and soluble reactive phosphorus (SRP)-P for three pelagic fish taxa common in Arctic Alaskan lakes: Arctic grayling and lake trout (2005 and 2007) and Arctic char (2008). Owing to the logistical difficulty in measuring excretion rates in situ, we only measured fish excretion rates for Arctic grayling (n = 31) and lake trout (n = 15) in Toolik Lake, and excretion rates for Arctic char (n = 2) were only measured in Lake Fog-2. Fish used in the measurement of excretion rates ranged in dry mass from 9 to 120 g for Arctic grayling, 18–190 g for lake trout, and 2–160 g for Arctic char. We collected Arctic grayling and lake trout using five-panel experimental gill nets, set for approximately 15 min intervals, thus minimizing the time fish spent entangled in the net. We used fyke nets to capture fish in Lake Fog-2 because of the difficulty in capturing Arctic char with gill nets. Regardless of capture method, methods used to measure excretion were identical for all fish species. After we collected water samples for nutrient analyses, fish were weighed (g), measured (total length, mm), and released back into the lake.

Prior to setting our nets, we filled 10 L sealable Ziploc plastic bags with 8 L of surface water that had been strained though 80 μm Nitex mesh to remove mesozooplankton. Bags were kept in a lightproof cooler to reduce warming and nutrient uptake by phytoplankton. When fish were captured they were immediately placed in a prefilled bag and incubated in the lake for ~0.5 to 1 h. Bags were suspended from a surface float and incubated at ambient epilimnetic water temperatures, usually between 10 and 15 °C, and water temperature within each bag was recorded before and after incubation.

For all of our nutrient excretion measurements, we had treatment (containing fish) and control (no fish) bags and initial (T_0) and final (T_1) sampling events. We used lake surface water for our incubations, and because the water source was from a well-mixed pool we only took T_0 nutrient samples from no fish bags. After incubation, however, we collected T_1 samples from all fish and no fish bags. We attributed the change between T_1 and T_0 nutrient concentrations in no fish bags to processes unrelated to fish excretion (e.g., photosynthetic uptake or microbial mineralization) and subtracted the difference from the change in nutrient concentrations of fish bags (see Nutrient Analysis section).

We collected all T_0 and T_1 nutrient samples in fish and no fish bags by filling a 140 cm^3 syringe with water from the respective bag, purging the initial draw, and forcing a second syringe full of water through 0.45 μm pore size, sterilized, mixed cellulose ester (MCE) membrane filter into an acid-washed, lightproof, high density polyethylene (HDPE) bottle. Bottles were placed in a cooler for less than 3 h, then returned to Toolik Field Station and frozen at –5 °C until analyzed for nutrient concentrations (see below).

Zooplankton excretion rates

During the summer of 2007, we experimentally measured zooplankton NH_4^+ and SRP excretion rates using temperature-controlled incubation chambers at Toolik Field Station. Initial attempts to manually separate small-bodied zooplankton taxa under a microscope resulted in high mortality of all species. Therefore, we combined small-bodied zooplankton for excretion measurements and used zooplankton from Lake S-11 (68°37.789′N, 140°38.922′W), located approximately 0.75 km from the western shore of Toolik Lake, as the source community for all of our zooplankton excretion experiments. The zooplankton community in Lake S-11 has relatively high abundances of the large-bodied copepod *H. septentrionalis* and cladoceran *D. middendorffiana*. In addition, the small-bodied zooplankton community is composed entirely of the copepods *D. pribilofensis* and *C. scutifer* (Kling et al. 1992). Because zooplankton were collected from one source community, as with fish, experimentally measured excretion rates were assumed to be applicable to all lakes in the study.

We collected zooplankton for excretion measurements by taking six replicate tows from the deepest part of Lake S-11 (~10 m) to the surface using a 30 cm diameter; 80 μm mesh size plankton net. Zooplankton tows were combined in a single 1 L low-density polyethylene (LDPE) bottle and immediately returned to Toolik Field Station.

Table 1. Depth, surface area, volume, watershed area, watershed to lake surface area (W:L) ratio, and fish community in six study lakes.

Lake	Latitude–longitude	Maximum depth (m)	Surface area (m^2)	Volume (m^3)	Watershed area (m^2)	W:L	Fish community
NE-12	68°39.730′N; 149°37.211′W	17	74 500	566 000	1 250 000	16.8	Arctic grayling, lake trout
GTH 86	68°37.886′N; 149°25.020′W	10	34 000	119 500	1 400 000	41.2	Arctic grayling
Fog-2	68°40.814′N; 149°05.779′W	20	56 500	469 000	460 000	8.1	Arctic char
GTH 114	68°40.706′N; 149°13.778′W	6	39 500	87 000	592 000	6.8	None
E-4	68°37.194′N; 149°29.656′W	4	39 500	82 500	461 000	5.6	None
GTH 99	68°29.891′N; 149°35.863′W	4	7 000	14 500	130 000	18.6	None

At Toolik Field Station we initially separated large and small-bodied zooplankton by straining the sample through 500 μm mesh size Nitex bolting cloth. The large-bodied species, *D. middendorffiana* and *H. septentrionalis*, were rinsed back into approximately 1 L of water and divided into two separate treatments using a Folsom plankton splitting wheel. We then visually separated the *D. middendorffiana* individuals from the *H. septentrionalis* treatment and vise versa using a pair of fine tip forceps, thus minimizing the handling of experimental organisms in each treatment. The small-bodied species, *D. pribilofensis* and *C. scutifer*, were too small to separate (described above), and we combined these two taxa as one treatment. We prepared the small-bodied copepod treatment by straining the remaining water sample, after removal of *D. middendorffiana* and *H. septentrionalis*, through 153 μm Nitex mesh. Once separated into treatments (hereafter Copepods, Daphnia, and Heterocope), we divided each treatment into four replicates using a Folsom plankton splitting wheel. Two replicates from the Heterocope treatment and one replicate from the Copepod treatment were lost during handling, and we had $n = 3$, 4, and 2 replicates for the Copepod, Cladoceran, and Heterocope treatments, respectively. Replicates from each treatment were strained from Lake S-11 water and rinsed into 250 mL volume LDPE bottles with surface water from Toolik Lake that had been previously strained through 80 μm Nitex mesh. Bottles were then filled to a total volume of 200 mL with the same strained surface water from Toolik Lake. We incubated bottles in a temperature controlled facility at Toolik Field Station for ~0.5 h at 15 °C for the Copepod and Daphnia treatments; however, because of a temperature constraint in the incubation facility we were forced to incubate the Heterocope treatment at 19 °C.

As with fish excretion experiments, we had both treatment (containing zooplankton) and control (no zooplankton) bottles and two sampling events, an initial T_0 and final T_1. We attributed the changes between T_0 and T_1 concentrations of NH_4^+ and SRP in the no zooplankton bottles to processes unrelated to zooplankton excretion and subtracted the difference from zooplankton bottles (eq. 2). Because Toolik Lake surface water was considered to be a well-mixed pool, we only took T_0 nutrient samples from no zooplankton bottles and assumed they represented background concentrations of NH_4^+ and SRP in zooplankton bottles as well.

After the incubation we used a 140 cm^3 syringe, with 80 μm Nitex mesh covering the intake to exclude zooplankton, to collect a T_0 water sample from all zooplankton and no zooplankton bottles. An initial ~10 mL of water was drawn to rinse the syringe, then purged. A second ~100 mL of water was then drawn into the syringe and forced through a 0.45 μm pore size, sterilized MCE membrane filter into an acid-washed, lightproof, HDPE bottle. The water samples were immediately frozen at –5 °C until concentrations of NH_4^+ and SRP were analyzed (see below).

Following the collection of water for nutrient analysis, we preserved the zooplankton in each treatment with sugar-buffered (concentration of 100 $g \cdot L^{-1}$) Lugol's solution and calculated the zooplankton biomass. Zooplankton biomass was calculated by first measuring the length of 10 individuals from each taxon using the ocular micrometer in a dissecting microscope and then counting the remaining zooplankton in the sample. We converted the length of each measured individual (mm) into dry mass (g) using length–weight regressions for each species (Burkart 2007). Mean biomass of the 10 measured individuals from each taxon was used as the average individual biomass in each respective population. We then calculated total zooplankton biomass in each treatment (g) by multiplying the average individual mass by total individuals.

Nutrient analysis

In 2005, we analyzed concentrations of NH_4^+ fluorometrically (Holmes et al. 1999) on a Turner Designs 10-AU field fluorometer, and SRP concentrations were analyzed colormetrically (Strickland and Parsons 1972) on a Shimadzu UV-Mini 1240 spectrophotometer. However, in 2007 and 2008 we shipped our frozen nutrient samples to Utah State University, and NH_4^+ and SRP concentrations were analyzed on a Astoria-Pacific 300 series flow injection analyzer following methods outlined in Eaton et al. (2005).

For all fish and zooplankton nutrient samples, we calculated mass-specific NH_4^+-N or SRP-P excretion rates using the following equation:

$$(1) \qquad E_M = \frac{(\Delta C_{\text{treatment}} - \Delta C_{\text{control}})V_c}{tm}$$

where E_M equals mass-specific excretion rates of either

NH_4^+-N or SRP-P ($\mu g \cdot g^{-1} \cdot day^{-1}$) for each fish or zooplankton treatment, $\Delta C_{treatment}$ is the change in concentrations ($\mu g \cdot L^{-1}$) of NH_4^+-N or SRP-P in fish and zooplankton treatments, $\Delta C_{control}$ is the change in NH_4^+-N or SRP-P concentrations in control samples without fish or zooplankton, V_c is the volume of water in the incubation container (litres), t is the duration of the incubation (days), and m is the dry mass (g) of either fish or zooplankton in the respective treatment. We also calculated the individual excretion rate for each treatment (individual fish and zooplankton) by multiplying E_M by the mass per individual in each treatment ($g \cdot individual^{-1}$). In control samples the change in NH_4^+ and SRP concentrations between T_0 and T_1 was always near zero. After calculating mass-specific excretion rates, we corrected the rates for the variation in temperature between different incubations during excretion measurements. We corrected all excretion rate measurements to a reference temperature of 15 °C and used temperature coefficients (Q_{10}) of 2.0 for fish N and P mass-specific excretion rates (Vanni 2002) and 2.0 and 1.9 for zooplankton N and P excretion rates, respectively (Ikeda et al. 2001). Excretion rates were corrected using the following equation:

$$(2) \quad E_M(15) = E_M(I) Q^{\frac{(15-I)}{10}}$$

where $E_M(15)$ is the mass-specific excretion rate at 15 °C, $E_M(I)$ is the mass-specific excretion rate at the respective incubation temperature, Q is the respective Q_{10} taken from the literature, and I is the incubation temperature for each excretion measurement (Haefner 2005).

Fish biomass

We determined fish biomass in lakes NE-12, GTH 86, and Fog-2 by first estimating fish abundance from multiyear mark–recapture data that were available for these lakes from 2005 to 2007. We pooled the 3 years of mark–recapture data to calculate a single estimate of fish abundance for each lake used in this study. During the mark–recapture study, fish were captured using a combination of angling, gill nets, and ice fishing, and efforts were made to recapture 30 marked individuals of each species in each lake. Gill nets were used to capture Arctic grayling and lake trout and were deployed for approximately 0.5 h intervals to minimize fish mortality. Shoreline angling was used to capture Arctic grayling and lake trout during these intervals. Arctic char are difficult to catch using the previously described techniques, and fish were captured in Lake Fog-2 by angling under the ice during the early spring. The previously described techniques would not have been effective for capturing juvenile or young-of-the-year fish smaller than approximately 90 mm, and there was no data available to estimate the abundance of fish from size classes < 90 mm. However, several studies have found that predation pressure by piscivorous adult fish constrains the distribution of juvenile fish to littoral habitats in these lakes (McDonald et al. 1992, 1996; Keyse et al. 2007), and as such we were comfortable excluding juvenile fish from estimates of pelagic nutrient regeneration.

Regardless of technique, when fish were captured they were weighed (g), measured (total length, mm), marked using a passive integrated transponder (PIT) tag, and released back into the lake. We used the Schnable method for calculating fish abundance from multiple mark–recaptures trials within a study period using the following equation:

$$(3) \quad \widehat{N} = \frac{\sum_t (C_t M_t)}{\sum_t R + 1}$$

where $\widehat{N}$ is the population estimate of fish within a lake, C_t is the number of fish captured during trial t, M_t is the number of marked fish at the beginning of trial t, and R_t is the number of marked fish captured during trial t (Krebs 1999). For the estimates of fish biomass, we assumed that dry mass was 20% of wet mass (Ricker 1968). Following our determination of fish abundance, we calculated fish biomass concentration ($g \cdot m^{-3}$) by first dividing fish captured into 50 g size classes. We then assumed that the size distribution of fish captured during the mark–recapture study represented the population as a whole and estimated the abundance of fish in each 50 g size class. Next we multiplied $\widehat{N}$ from each size class by the median dry weight (g) per class, then summed the size class biomass estimates from each species in each study lake and divided by the lake volume (m^3). We used these same estimates of fish biomass in each 50 g size class when calculating whole-lake estimates for fish N and P recycling (described below).

Zooplankton biomass

We collected zooplankton samples for biomass calculation in fish and fishless lakes within a 5-day time period during early July 2008. Zooplankton samples were collected after the lakes were thermally stratified when zooplankton communities were likely near peak biomass (Johnson et al. 2007). Zooplankton were collected by taking three replicate tows from each lake with a 30 cm diameter, 80 µm mesh plankton net from ~1 m off the bottom, in the deepest location, to the surface. We immediately preserved zooplankton samples in sugar-buffered ($100 \ g \cdot L^{-1}$) Lugol's solution and returned them to Toolik Field Station for biomass analysis.

The biomass of zooplankton in each tow was estimated using the same methods applied during zooplankton excretion experiments (described above). However, we calculated tow biomass concentration ($g \cdot m^{-3}$) by dividing the biomass of zooplankton in each tow by the tow volume.

Whole-lake nutrient recycling

We used the mass-specific excretion rates for fish and zooplankton to calculate whole-lake estimates of N and P recycling from each consumer group. For fish we used a log-linear regression model of fish dry mass by mass-specific excretion rate to calculate a mass-specific excretion rate for each 50 g size class. We then used each 50 g size class as a separate fish group when we calculated whole-lake nutrient recycling from fish. Because we captured only two Arctic char for excretion measurements, we combined Arctic char with lake trout. These two species are evolutionarily close relatives, within the same genus *Salvelinus*, and fill a similar trophic niche in Arctic Alaska lakes (Hershey et al. 1999). We calculated whole-lake estimates of N and P recycling from fish (50 g size classes) and zooplankton (Copepod, Cladoceran, Heterocope) groups in each lake using the following equation:

$$(4) \quad E_L = \frac{\sum (E_{M,i} \times B_i)}{1000}$$

where E_L equals whole-lake NH_4^+-N or SRP-P recycling rates ($\mu g \cdot L^{-1} \cdot day^{-1}$) from fish and zooplankton, and $E_{M,i}$ and B_i equals the respective mass-specific excretion rate (eq. 1) and biomass concentration ($g \cdot m^{-3}$) for each consumer group (i).

After we calculated whole-lake estimates of N and P recycling from fish and zooplankton consumer groups, we determined the importance of recycled nutrients for phytoplankton production by comparing rates of N and P recycling from fish and zooplankton with rates of N and P uptake during primary productivity (Vanni 2002). By using this method, we calculated the potential, not actual, proportion of phytoplankton nutrient demand supplied by fish and zooplankton nutrient recycling. We were comfortable using this method because of the high frequency of N and P colimitation in Arctic Alaskan lakes (Levine and Whalen 2001) and low concentrations of inorganic N and P, which are often at or below detection limits (Kling et al. 1992).

Phytoplankton nutrient demand

We used rates of pelagic primary productivity ($\mu g \ C \cdot L^{-1} \cdot day^{-1}$) that had been previously measured using radioisotope (^{14}C) uptake methods as estimates of phytoplankton N and P demand (Whalen et al. 2006, 2008) and as described here. In Lake Fog-2, water samples for primary productivity analysis were collected with a Van Dorn sampler at depths of 0, 1, 3, 5, 8, and 12 m on two dates each in 2000 (14 July and 4 August) and 2005 (30 June and 7 July). Pelagic photosynthesis was determined using 24 h in situ ^{14}C incubations as described by Miller et al. (1986), except that 70 mL bottles were used for incubations, and samples were incubated at equivalent depths in Toolik Lake because of logistical constraints. Volumetric production measurements ($\mu g \ C \cdot L^{-1} \cdot day^{-1}$) at discreet measurement depths were extrapolated to average whole-lake production by multiplying each point measurement by the volume of its stratum, using midpoint interpolation between measurement depths to determine depths of stratum cutoffs and lake bathymetry to calculate the volume in each stratum. Total production in each stratum was then summed across strata to find whole-lake carbon uptake, and this was divided by lake volume (L) and averaged across sampling dates to give average whole-lake production ($\mu g \ C \cdot L^{-1} \cdot day^{-1}$).

The relationship between photosynthesis and irradiance (PI curve) was used to estimate phytoplankton primary production in lakes GTH 86 and E-4 in 2008. PI curves were constructed by measuring $H^{14}CO_3^-$ uptake in a "photosynthetron" (Lewis and Smith 1983). A 250 mL sample of lake water from the depth corresponding to 25% of surface photosynthetic photon flux density was amended with $H^{14}CO_3^-$ (final activity 265 $Bq \cdot mL^{-1}$), and 21 aliquots of 8 mL were dispensed into scintillation vials in the incubator. Triplicate aliquots were dispensed into vials containing 100 µL of borate-buffered formalin as zero-time controls. Radioactivity of the 250 mL sample was assessed by subsampling 100 µL into 2 mL of 1 $mol \cdot L^{-1}$ NaOH. Light in the incubator was provided by a Cool-Lux projector lamp and was measured with a Biospherical Instruments QSL-100 4π quantum sensor inserted into each scintillation vial. Samples were incubated for 0.75 to 1.5 h at the temperature of collection and then killed by addition of 0.1 mL of 1 $mol \cdot L^{-1}$ HCl. Unassimilated $H^{14}CO_3^-$ was purged from each sample by bubbling with He for 0.5 h. Samples were amended with 10 mL of scintillation cocktail (Aquasol II) and assayed for radioactivity.

The relationship between photosynthesis and irradiance was assessed by directly fitting the data (quasi-Newton method; Systat statistical package, Systat Software Inc., Chicago, Illinois) to eq. 5 (Platt et al. 1980), which assumes no photoinhibition:

$$(5) \quad P^B = P_m^B(1 - e^{-\alpha I})$$

where P^B is the photosynthetic rate normalized to chlorophyll a (mg C·mg chl $a^{-1} \cdot h^{-1}$); P_m^B is the maximum potential light-saturated rate of photosynthesis (mg C·mg chl $a^{-1} \cdot h^{-1}$); I is the photon flux density ($mol \cdot m^{-2} \cdot h^{-1}$); and $\alpha = \alpha I / P_m^B$. Here, α is the initial linear increase in photosynthesis with increasing irradiance (mg C·mg chl a^{-1}·mol photons$^{-1} \cdot m^{-2}$).

E-4 and GTH 86 had similar light extinction coefficients ($0.93 \ m^{-1}$) and α was 20 and 13.9 for the respective lakes. We calculated P^B at 1 m depth strata in the euphotic zone of each lake, defined as the depth of light penetration to 1% of surface photosynthetic photon flux density (PPFD), by using the average July surface PPFD (Weather Data Query, University of Alaska Fairbanks, Institute of Arctic Biology, Toolik Field Station, http://toolik.alaska.edu/edc/weather/data_query.php, February 2010), corrected for 5% surface reflection of incident light (Carignan and Planas 1994), and attenuated for each depth strata.

To calculate lake volume-based primary production ($\mu g \ C \cdot L^{-1} \cdot day^{-1}$) in the euphotic zone, we assumed that chlorophyll a concentration was 2 $\mu g \cdot L^{-1}$ at each depth strata, multiplied P^B by chlorophyll a concentration, integrated the result between depth strata, then summed the integrated (P^B·chlorophyll a) value for each depth strata and divided by the depth of the euphotic zone.

Owing to the ultra-oligotrophic conditions that typify lakes in this region, algal C:N:P ratios are usually higher than the Redfield ratio. Therefore, we converted water column carbon uptake to nutrient demand by using an average phytoplankton C:N:P, by mass, for lakes in this region of 77:9:1 (LaPerriere et al. 2003). To calculate the percentage of phytoplankton nutrient demand potentially supported by consumer nutrient recycling in our study lakes, we divided the rates of NH_4^+-N or SRP-P recycled by fish and (or) zooplankton in each lake by the respective estimates of N and P required for observed rates of phytoplankton production.

Statistical analyses

We used Student's t tests to compare the differences in mean zooplankton biomass and mean zooplankton length between fish and fishless lakes. For the analysis of zooplankton length, we used the mean length (mm) of individual zooplankton in samples collected for the calculation of zooplankton biomass and weighted the mean by the density ($individuals \cdot L^{-1}$) of each taxa in the six study lakes. For comparisons of both zooplankton biomass and individual length, we used the study lakes as replicates and compared

the means from the three fish lakes (NE-12, GTH 86, Fog-2) with the means of three fishless lakes (E-4, GTH 114, GTH 99) (Table 1). Values of zooplankton biomass and length were log-transformed to meet the equal variance assumption for statistical analysis.

We also quantified the zooplankton community composition in each study lake to determine if the presence of a fish community shifted the zooplankton communities to copepod compared with cladoceran dominance. We analyzed the percentage of total zooplankton biomass comprised by each species in the six study lakes and qualitatively compared the percentage of copepod and cladoceran taxa between fish and fishless lakes. All statistical analyses were performed using SAS statistical program JMP 7.0.1 (SAS Institute Inc., Cary, North Carolina).

Results

We measured rates of N and P excretion by fish and zooplankton consumer groups to determine the contribution of recycled nutrients to primary productivity. In addition, we investigated the direct contribution of fish excretion to the recycled nutrient pool and compared it with the potential for fish to have an indirect control on zooplankton nutrient recycling by effecting zooplankton size, biomass, and community composition.

Consumer excretion rates

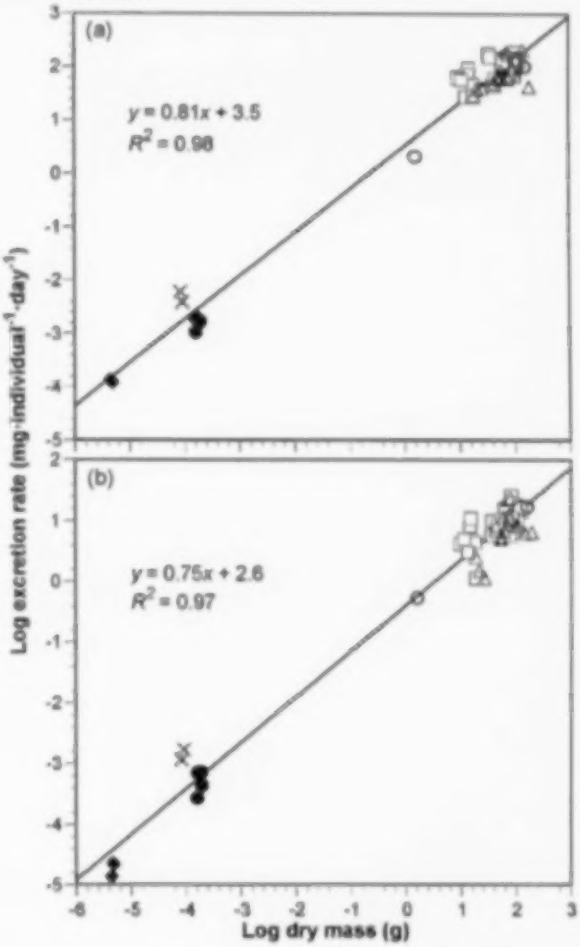
Nutrient excretion rates for both NH_4^+ and SRP increased with organism body size. However, the slopes of the log-linear relationships were less than one: 0.81 and 0.75 for NH_4^+ and SRP, respectively (Fig. 1). This illustrates that mass-specific excretion rates decreased with body size for the fish and zooplankton taxa included in this study, and the decrease was similar to the 3/4 scaling relationship between body size and metabolism (Brown et al. 2004).

Consumer biomass and community composition

Estimates of fish abundance were greater in the two relatively large volume study lakes with fish, NE-12 and Fog-2, compared with the relatively small volume fish lake GTH 86 (Fig. 2). The populations of Arctic grayling in lakes NE-12 and GTH 86, and Arctic char in lake Fog-2 had the highest frequency of individuals in the size classes between 50 and 200 g (Figs. 2*b*, 2*c*, 2*d*). In contrast, the population of lake trout in lake NE-12 had a relatively even distribution of individuals between the 50 and 1500 g size classes (Fig. 2*a*). Fish biomass estimates ($g \cdot m^{-3}$) were similar and had overlapping 95% confidence intervals for all three study lakes with fish (Fig. 3), indicating that fish abundance and biomass were largely a function of lake volume.

Zooplankton biomass was significantly lower in lakes where fish were present ($p = 0.04$, $t = 2.98$, df = 4). The average zooplankton biomass was 0.14 $g \cdot m^{-3}$ in lakes without fish compared with only 0.03 $g \cdot m^{-3}$ in lakes where fish were present, an almost fivefold difference between fish and fishless lakes (Fig. 4). The weighted average of individual zooplankton length was lower in lakes with fish (mean = 0.9 mm) compared with fishless lakes (mean = 1.3 mm); however, this difference was not statistically significant ($p = 0.18$, $t = 1.61$, df = 4) (Fig. 4).

Fig. 1. Log–log scatterplot of (*a*) NH_4^+-nitrogen and (*b*) SRP-phosphorus individual excretion rates by individual dry mass for fish (open squares = Arctic grayling, open triangles = lake trout, open circles = Arctic char) and zooplankton (solid diamonds = copepods, solid circles = cladocerans, times symbol = heterocope) consumer groups.



In addition to reducing total zooplankton biomass, fish also had an effect on zooplankton community composition. Copepods were always >50% of the zooplankton community biomass in lakes with fish, while cladocerans comprised >60% of the zooplankton community biomass in lakes without fish (Fig. 5). Fish did not completely eliminate large-bodied zooplankton taxa, however, and we found relatively high proportions of the large-bodied species *D. middendorffiana*, *H. gibberum*, and *H. septentrionalis* in zooplankton communities of two lakes with fish, Fog-2 and GTH 86 (Fig. 5). In addition, the zooplankton communities in two fishless lakes, GTH 114 and E-4, had relatively high proportions of the small-bodied species *D. pribilofensis* (Fig. 5).

Consumer excretion and phytoplankton uptake

Fish excretion of N and P was low and contributed minimally to phytoplankton nutrient demand in our study lakes.

Fig. 2. Frequency of fish captured during mark–recapture trials broken up into 50 g (wet mass) size classes. Panels include an abundance estimate and upper 95% and lower 95% confidence intervals for lake trout (open bars), Arctic grayling (black bars), and Arctic char (grey bars) in (*a*) Lake NE-12, (*b*) Lake NE-12, (*c*) Lake GTH 86, and (*d*) Lake Fog-2.

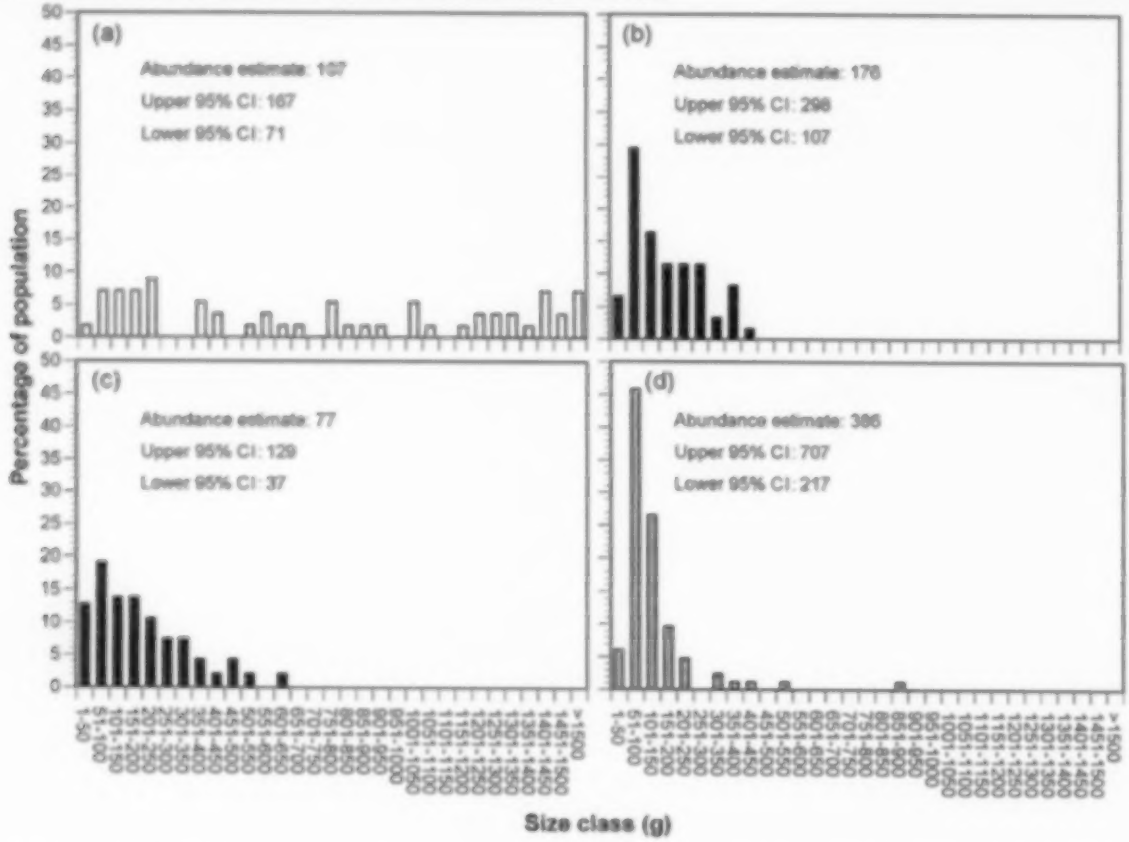


Fig. 3. Estimates of total lake trout (open bar), Arctic grayling (black bar), and Arctic char (grey bar) biomass in the three study lakes containing fish populations. Error bars are 95% confidence intervals.

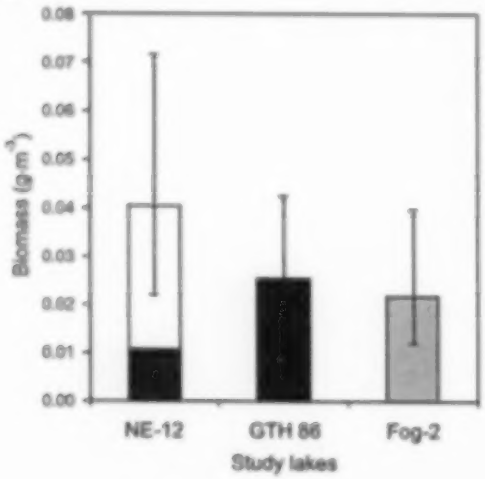


Fig. 4. Average individual length, weighted by species density, and total zooplankton biomass in lakes where fish were present (shaded bars) and absent (open bars). Data were log-transformed to meet assumptions for statistical analyses. An asterisk (*) indicates statistically significant ($p < 0.05$) difference between means. Error bars are ± 1 standard error.

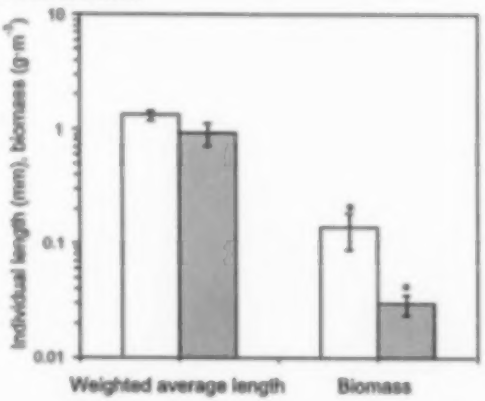
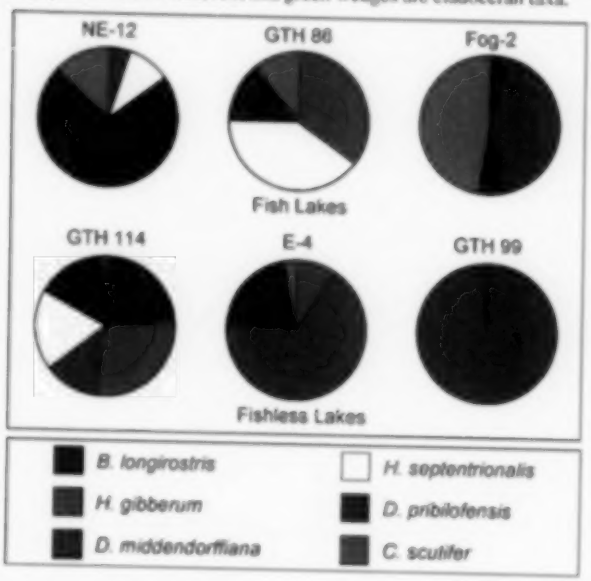


Fig. 5. Zooplankton species community composition in fish and fishless lakes. Wedges are the percentages of total zooplankton biomass comprised by individual taxa. Black, white, and gray wedges are copepod taxa; and blue, red, and green wedges are cladoceran taxa.



Fish supplied between <1% and ~5% of both N and P demand in the three study lakes with fish populations (Table 2). In contrast, zooplankton excretion of N and P was greater than fish excretion by approximately two orders of magnitude in the six study lakes (Table 2). Nutrient excretion by zooplankton showed the potential to be a large source of N and P for phytoplankton production, but also showed higher variability between lakes. Among lakes with fish populations, zooplankton nutrient recycling in lakes NE-12 and Fog-2 could supply ~132% and ~78% of N demand, as well as ~198% and 135% of P demand for phytoplankton production, respectively. Zooplankton nutrient excretion relative to phytoplankton demand was lower in lake GTH 86, but still accounted for ~19% and ~37% of phytoplankton N and P demand, respectively (Table 2). Among the fishless lakes, zooplankton excretion in E-4 and GTH 114 supplied similar proportions of phytoplankton N (19% and 23%) and P (41% and 48%) demand, compared with the other fishless lake, GTH 99, which had the potential to supply ~43% of phytoplankton N demand and ~125% of P demand (Table 2).

Results of these analyses indicated that direct excretion of N and P by fishes had minimal impact on phytoplankton nutrient demand. Excretion of P by zooplankton was sufficient to provide all of the P needed for phytoplankton growth in three of the six study lakes. However, excretion of N by zooplankton was less important than P in providing nutrients for phytoplankton.

Discussion

There is a growing body of literature that shows excretion of bioavailable N and P from consumer groups can have a strong bottom-up control on phytoplankton production rates (Vanni 2002). However, the circumstances under which autogenic nutrient recycling is a relatively large component of phytoplankton nutrient budgets (Attayde and Hansson 2001; Villar-Argaiz et al. 2001; Verant et al. 2007) and when it is negligible compared with other nutrient sources (Wurtsbaugh 2007) are still unclear. The results from our study support those from other low-productivity systems that have found nutrients recycled by zooplankton could account for a significant proportion of phytoplankton nutrient demand (Nakashima and Leggett 1980; Villar-Argaiz et al. 2001; Sarnelle and Knapp 2005). Indeed, the range of P recycling rates from zooplankton in our study closely overlapped mid-season rates of zooplankton P excretion (0.09–2.5 μg $P \cdot L^{-1} \cdot day^{-1}$) reported by Villar-Argaiz et al. (2001) from La Caldera Lake during 1996 and 1997, and in both studies nutrient recycling by zooplankton could account for >100% of phytoplankton demand.

The importance of fish excretion for primary production has also been shown to increase in concert with lake productivity (Drenner et al. 1996; Persson 1997; Vanni et al. 2006), and in oligotrophic systems the contribution of fish excretion to pelagic nutrient cycles can be minimal (Nakashima and Leggett 1980; Schindler et al. 1993). In contrast, Schindler et al. (2001) concluded that regeneration of P by trout in oligotrophic mountain lakes, while small (average 2.3 mg $P \cdot m^{-3} \cdot year^{-1}$), was important in the pelagic P cycle because the origin of P excreted by fish was primarily of benthic or terrestrial origin and thus was a source of "new" nutrients. Our measurements of P excretion from fish were nearly identical to those in Schindler et al. (2001), and fish in Arctic Alaskan lakes also obtain the majority of their food resources from benthic sources (Sierszen et al. 2003; O'Brien et al. 2005). We agree with the conclusion of Schindler et al. (2001) that fish excretion is a source of new nutrients, and fish in our study likely translocated N and P to epilimnetic waters from benthic habitats. However, the relatively low proportion of phytoplankton nutrient demand (between <1% and 10%) attributable to the direct excretion of N and P by fish in our study leads us to also agree with the findings of Hudson et al. (1999) that fish excretion is relatively insignificant when compared with planktonic nutrient regeneration.

Although we found that relative to zooplankton, direct nutrient excretion by fish was minimal, fish had the potential to have indirect controls over nutrient recycling rates. Our estimates of zooplankton biomass in lakes with fish were significantly lower compared with lakes where fish were absent, and because of the lower zooplankton biomass, lakes with fish generally showed lower rates of zooplankton N and P excretion compared with lakes without fish (Fig. 6). The reduction of zooplankton biomass by fish predation is well documented (Carpenter and Kitchell 1988; Rudstam et al. 1993; Parker and Schindler 2006), and we concluded that not only was direct excretion from fish relatively inconsequential, but that fish also had a negative impact on zooplankton nutrient recycling.

In contrast, we did not observe a similar significant reduction in the average length of zooplankton in lakes where fish were present, even though this relationship has been observed in many lake ecosystems (Brooks and Dodson 1965; Parker and Schindler 2006). Several studies have shown that

Table 2. Consumer biomass, mean individual zooplankton length, primary productivity, phytoplankton nutrient demand, consumer nutrient excretion rates, and percentage of nutrient demand supplied by each consumer group for the six study lakes.

Lake	Consumer	Biomass ($g \cdot m^{-3}$)	Mean length (mm)	Primary productivity ($\mu g\ C \cdot L^{-1} \cdot day^{-1}$)	Phytoplankton nutrient demand ($\mu g \cdot L^{-1} \cdot day^{-1}$)		Consumer nutrient excretion ($\mu g \cdot L^{-1} \cdot day^{-1}$)		% of nutrient demand	
					Nitrogen	Phosphorus	Nitrogen	Phosphorus	Nitrogen	Phosphorus
NE-12	Fish	0.041	—	8.40[a]	1.03	0.11	0.050	0.005	5.1	4.9
	Zooplankton	0.043	0.8				1.29	0.22	131.9	197.5
GTH 86	Fish	0.026	—	56.4	6.59	0.73	0.051	0.006	<1	<1
	Zooplankton	0.038	1.4				1.25	0.27	19.0	36.7
Fog-2	Fish	0.012	—	4.57	0.53	0.06	0.027	0.003	5.1	4.6
	Zooplankton	0.021	0.7				0.42	0.08	77.8	134.9
GTH 114	Fish	—	—	52.80[a]	6.17	0.69	—	—	—	—
	Zooplankton	0.061	1.6				1.40	0.33	22.7	48.0
E-4	Fish	—	—	81.6	9.53	1.06	—	—	—	—
	Zooplankton	0.129	1.1				1.81	0.44	19.0	41.3
GTH 99	Fish	—	—	45.60[a]	5.33	0.59	—	—	—	—
	Zooplankton	0.232	1.3				2.30	0.74	43.1	125.1

[a]From Whalen et al. 2006.
[b]From Whalen et al. 2008.

Fig. 6. (*a*) NH_4^+-nitrogen and (*b*) SRP-phosphorus excretion rates ($mg \cdot m^{-3} \cdot day^{-1}$) for fish (squares) and zooplankton (circles) consumer groups compared with phytoplankton nutrient uptake (grey bars). Error bars on fish and zooplankton excretion rates are calculated from 95% confidence intervals around estimates of consumer biomass.

indirectly increasing recycling rates via changing zooplankton body size can be the largest impact fish have on internal nutrient recycling (Bartell 1981; Schindler et al. 1993; Attayde and Hansson 2001). However, we did not find evidence for this indirect effect of fish in our current study. In Arctic Alaskan lakes, two commonly found large-bodied zooplankton species, *H. septentrionalis* and *H. gibberum*, have effective defenses against fish predation (O'Brien et al. 1979*a*, 1979*b*), and these two taxa comprised ~75% of the zooplankton biomass in the fish lake GTH 86. The mean length of individual zooplankton in Lake GTH 86 was the second highest measured in the six study lakes and was the reason we did not find a significant difference in zooplankton length between fish and fishless lakes. However, O'Brien et al. (2004) found in a survey of Arctic Alaskan lakes that individual *Daphnia* were smaller in lakes with fish, suggesting that a significant impact of fish on zooplankton mass-specific excretion rates may have become apparent with a larger number of lakes in this study.

Although beyond the scope of this research, fish may have had an indirect effect on the stoichiometry, and subse-

quent availability, of recycled N and P in these lakes. There is considerable disparity in the ratio of N:P recycled by copepods and cladocerans because of a discrepancy in the nutrient demand for somatic growth and reproduction (Sterner and Elser 2002). In our study, zooplankton biomass in lakes with fish was composed of 53%–95% copepods, while zooplankton biomass in lakes without fish was 65%–99% cladoceran. Differential N:P recycling by copepods and cladocerans can be an important driver of N versus P limitation for phytoplankton growth (Elser et al. 1988, 1995; Johnson 2009), and fish in Arctic Alaskan lakes may control phytoplankton productivity indirectly through this mechanism.

There are key issues with our study that should be noted: the extrapolation of fish and zooplankton excretion rates to additional lakes, the low number of excretion measurements made with Arctic char, and the fact that we did not filter phytoplankton and bacteria from the incubation water prior to fish and zooplankton excretion measurements. It was not feasible to transport the equipment required to incubate fish within the lake to some of our study lakes, and as such we were forced to make our excretion measurements in Toolik Lake and Fog-2. Verant et al. (2007) showed that fish density and diet explained the greatest amount of variability in community measurements of N and P excretion by fish, larger than community composition, water temperature, fish growth rate, and fish size. We used fish biomass within each lake to estimate community N and P excretion from fish and used 95% confidence intervals to calculate the upper and lower ranges of these measurements. In addition, there is very little interlake diet variability within fish species because lakes in this region are characteristically poor in food resources for fish (Hershey et al. 1999; Sierszen et al. 2003). As a result, we felt that using mass-specific excretion rates measured in Toolik Lake and Fog-2 in conjunction with estimates of fish biomass broken down into 50 g size classes from each study lake provided valid estimates of fish community excretion rates for each lake.

By comparison, zooplankton rates of N and P excretion are dependant on the taxon from which rates are measured (Elser and Urabe 1999). By measuring excretion rates separately for copepods and cladocerans, we were able to account for this variability. In addition, we standardized all excretion rate measurements to a temperature of 15 °C to correct for differences in temperatures during fish and zooplankton incubations. Because of these efforts, we felt comfortable extrapolating our measurements of fish and zooplankton excretion to the six lakes used in this study.

We had minimal success in capturing Arctic char for excretion measurements, and we combined the rates of N and P excretion measured from the two Arctic char captured with rates measured from lake trout. These two fish species function similarly in Arctic lake food webs (Hershey et al. 1999), and coupled with the findings of Verant et al. (2007), we feel combining excretion rates from these two species was valid.

It has been shown that failing to filter phytoplankton and bacteria from water used in measurements of consumer excretion rates can result in underestimates of rates because of nutrient uptake concurrent with remineralization or result in overestimates of rates because of lysis of algal cells during zooplankton feeding (Lehman 1980*a*, 1980*b*). The rates of N and P excretion measured in our study were likely influenced by both of these processes. However, algal concentrations and rates of nutrient uptake are very low in Arctic Alaskan lakes, and with short incubation times (between 0.25 and 0.5 h) we feel the potential confounding effects from nutrient uptake and cell lysis were minimal. Indeed, the rates of nutrient excretion we measured for fish and zooplankton in this study closely overlapped with rates reported in other studies (Ganf and Blazka 1974; Lehman 1980*a*; Schindler et al. 2001), and we feel confident about the robustness of our measurements.

Our research suggests that nutrient recycling from zooplankton communities can be an important source of N and P for phytoplankton production during the summer and that nutrient recycling within the water column needs to be considered in whole-lake nutrient budgets. By investigating lakes both with and without fish we were able to compare the direct and indirect effects of fish on rates of internal nutrient recycling. We found little support for the importance of nutrients excreted directly by fish, but found that fish can indirectly lower rates of N and P recycling by reducing total zooplankton biomass. More research is required, however, to clarify the sources of variability in primary production rates in this region. Bioavailability of N and P can be a key driver of primary production, and our research suggests that internal nutrient recycling should be considered with watershed nutrient loading as an important source of potentially limiting nutrients.

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Flow permanence affects aquatic macroinvertebrate diversity and community structure in three headwater streams in a forested catchment

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Abstract: Drying can be a common disturbance affecting macroinvertebrate communities in headwater streams. Whether intermittent and ephemeral streams have a lower diversity and (or) unique assemblage structure relative to physically similar and nearby perennial streams is still debated. We investigated changes in the diversity and assemblage composition of aquatic macroinvertebrates occupying debris dams in three headwater streams with a gradient of flow permanence (perennial, intermittent, and ephemeral) during a dry period in the austral summer of 2007 and a wet period in the spring of 2008. In the dry period, mean taxon richness and abundance in debris dams were lower in the intermittent and ephemeral streams than in the perennial stream, and the length of time without connected surface flow appeared to produce different patterns in community composition. However, during the wet period, mean taxon richness, abundance, and community composition of macroinvertebrates were very similar among the three streams. Hierarchical Bayesian modeling showed evidence for a strong effect of permanence on taxon richness, abundance, and evenness within debris dams. Taxa from the perennial stream were extremely efficient at colonizing seasonally dry nearby streams. Differences in assemblage structure between these temporary and permanent headwater streams may only arise seasonally and also appear related to flow permanence.

Résumé : La sécheresse peut être une perturbation qui affecte fréquemment les communautés de macroinvertébrés dans les cours d'eau d'amont. Il existe encore un débat à savoir si les cours d'eau temporaires et éphémères possèdent une diversité réduite et (ou) une structure de peuplement particulière par rapport aux cours d'eau permanents physiquement semblables et situés à proximité. Nous avons étudié les changements de diversité et de composition des peuplements de macroinvertébrés aquatiques dans les barrages de débris dans trois cours d'eau d'amont présentant un gradient de permanence du débit (permanent, intermittent et éphémère) durant une période de sécheresse pendant l'été austral en 2007 et une période de précipitations au printemps 2008. Durant la période sèche, la richesse moyenne en taxons et l'abondance dans les barrages de débris étaient plus basses dans les cours d'eau intermittent et éphémère que dans le permanent et la durée de la période sans écoulement continu en surface semble déterminer des patrons différents de composition de la communauté. Cependant, durant la période humide, la richesse moyenne en taxons, ainsi que l'abondance et la composition de la communauté de macroinvertébrés, sont très semblables dans les trois cours d'eau. Une modélisation hiérarchique bayésienne donne des indications d'un effet important de la permanence sur la richesse taxonomique, l'abondance et l'équitabilité dans les barrages de débris. Les taxons provenant du ruisseau permanent sont extrêmement efficaces pour coloniser les ruisseaux à sécheresse saisonnière situés à proximité. Les différences de structure de peuplement entre les cours d'eau d'amont temporaires et permanents peuvent n'exister que saisonnièrement et semblent reliées à la persistance de l'écoulement.

[Traduit par la Rédaction]

Introduction

Drying of stream ecosystems is a local and regional disturbance for stream biota (Lake 2003) and a major factor influencing macroinvertebrate assemblage structure (Williams 1996). The temporary absence of surface flow is particularly common in headwater streams (springs excluded), and these streams can be highly responsive to single precipitation

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events (Richardson and Danehy 2007). Temporary waters can be subdivided into those that are dry at times of the year that are more or less predictable (intermittent) and those that contain water on an unpredictable basis (ephemeral) (Williams 1996). High heterogeneity of the physical habitat in headwater streams (Gooderham et al. 2007) and highly variable local discharge (Gomi et al. 2002) mean that single catchments often contain headwater streams that are both permanent and temporary.

Poff and Ward (1989) argued that macroinvertebrate diversity should be low under conditions of intermittency, and Vinson and Hawkins (1998) found that most studies reported lower richness in temporary streams than in permanent streams and that richness generally increased with increasing flow duration. Conversely, some studies have found taxon richness in temporary streams to exceed that of perennial streams (Dieterich and Anderson 2000). Taxa inhabiting temporary streams require one or more strategies to cope with the onset of drying and may possess functional traits that allow them to persist under such conditions. These may include short or flexible life cycles, long-lived propagules, resistance to desiccation, good dispersal ability, or behavioral adaptations such as drifting or seeking refuge (Williams 1996; Humphries and Baldwin 2003; Bêche et al. 2006). Therefore, temporary streams may harbour unique or distinctive macroinvertebrate communities that differ from nearby perennial streams (Feminella 1996; Williams 1996). Taxa without these functional traits may not persist in temporary streams or may seek refuge on a seasonal basis and return during periods of increased hydrologic connectivity through aerial or overland dispersal or drift.

We investigated aquatic macroinvertebrate communities in three nearby and physically similar headwater streams that differed primarily in their level of flow permanence to determine (*i*) whether flow permanence affects diversity, (*ii*) whether flow permanence affects community composition, and (*iii*) which macroinvertebrate taxa (or groups of taxa) show the strongest response to flow permanence. We focused on seasonal drying (over one year), but the study was undertaken during a time of severe and prolonged drought affecting streams in southeastern Australia over the past decade (Rose et al. 2008; McGowan et al. 2009).

Materials and methods

Study area and design

The study was conducted in three unnamed first-order streams in the Wallaby Creek Designated Water Supply Catchment Area (37°4′S, 145°2′E). This 9965 ha closed catchment is located at the southern end of the Hume Plateau on top of the Great Dividing Range in Victoria, Australia (Ashton 2000), and is approximately 50 km northeast of the state capital, Melbourne. In the wider catchment, annual mean rainfall was 1207 mm over the period 1995–2006 (Martin et al. 2007), and elevation ranges from 600–800 m above sea level (asl). The vegetation is dominated by an ecologically mature, eucalypt forest that encompasses a mixture of Ecological Vegetation Classes (EVCs), including restricted pockets of Cool Temperate Rainforest, Wet Forest, Damp Forest, Shrubby Foothill Forest, and Riparian Forest (Frood et al. 2001). EVCs are the recognized units of the Victorian Government vegetation classification system and represent groupings of vegetation communities based on floristic, structural, and ecological features. The catchment area is closed to the general public and is primarily managed for protection of water quality and water supply (Parks Victoria 1998). At the time of this study, the area was largely undisturbed, but there are historic disturbances (mostly occurring in the mid- to late 1800s), including bushfire, aqueduct construction, logging, human settlement, and bullock grazing (Ashton 2000).

All studied streams are tributaries of Silver Creek and are within 2 km of each other. Streams are similar with respect to width (<0.5 m), gradient (very low, no riffles present in any of the study streams), drainage area (<1.4 km^2), EVC (Riparian Forest), geology (Chesney Vale granite), and soils (rich krasnozemic loam, which has a high organic matter content in the uppermost layers) but have a range of flow permanence from perennial to ephemeral. Permanency was calculated as the proportion of site visits in which a stream was flowing. Twelve site visits were made over a period of 26 months commencing three months before the first sampling period and concluding one week after the last sampling period. The perennial stream was flowing in 10 out of 12 visits (F (flow permanence) = 0.83), the intermittent stream was flowing in 7 out of 12 visits (F = 0.58), and the ephemeral stream was flowing on only one occasion (F = 0.08).

It was not possible to obtain replicates of each stream type, as there were no other streams in this catchment that displayed a high degree of permanency. Additionally, of the small number of headwater streams in the catchment (~10), several streams were inaccessible or had been artificially modified by the construction of small aqueducts. In light of this, we recognize that our findings cannot provide generalizations about patterns and processes in all temporary streams, but nonetheless, they can provide an insight into the patterns and processes occurring in this catchment, which is an important example of a mature and largely undisturbed forest with high biological integrity that would have been more widespread in the past.

Regional drying and drought history (1961–2008)

Ecological patterns and processes in streams (e.g., diversity patterns or life history strategies) may be correlated with long-term flow patterns (Lytle and Poff 2004) but also confounded by drying and drought events occurring at smaller temporal or spatial scales (Bêche and Resh 2007). To assess how representative flow conditions were during our study, we examined several decades (1961–2008) of daily stream flow data from a gauging station approximately 10 km northeast of the study site on King Parrot Creek at Flowerdale (station no. 405231) to determine whether the severity of the current drought was greater than in previous periods of drying or drought in the past half century. We calculated the cumulative streamflow deviation (in millimetres) for any run of consecutive wet or dry years. When there was a transition from an above-average or below-average streamflow year, we reset the deviation to zero.

Macroinvertebrate sampling

All study streams were located within the Riparian Forest

EVC. The structure of this EVC is generally open forest to tall woodland with a layered understory comprising a range of eucalypt species. The tall *Eucalyptus viminalis* is usually the dominant species and occurs with *Eucalyptus melliodora*, *Eucalyptus obliqua*, *Eucalyptus radiata* ssp. *radiata*, and *Eucalyptus ovata*. The dominance of eucalypt species beside these streams has considerable influence on in-stream habitat because, unlike many streams in North America and Europe, eucalypt species are not deciduous but have continuous leaf litter inputs throughout the year (with a peak in summer) and much slower rates of leaf breakdown (Pozo et al. 1997). This means that features such as leaf packs and debris dams are common habitats in temperate Australia streams and contribute significantly to the structural heterogeneity of low-gradient sand-bottom reaches. This is particularly true in small headwater streams where there is a high ratio of structural component size to stream width due to lack of stream competence to move material that has fallen onto the bed and banks (Gooderham et al. 2007).

Initial field observations of all headwater streams in the study area revealed only two dominant habitat types, sandy benthic areas and debris dams. We followed the definition of debris dams of Smock et al. (1989, p. 765) as "any wood > 5 cm in diameter in contact with sediment and spanning at least one-fourth of the channel. Also included were root masses from living riparian vegetation." The debris dams in the study streams generally were characterized by one larger piece of wood causing a partial obstruction of the channel and an accumulation of smaller pieces of wood, twigs, and leaves. Subsequent work for another study in the Silver Creek catchment quantified the previously observed dominance of the debris dam habitat type in the study area through records of the percentage of streambed in 30 m reaches occupied by debris dam habitats. The percentage of reach length occupied by debris dam habitats ranged from 33% to 53% in 2007 and from 13% to 73% in 2008 (A. Clarke, unpublished data). This study therefore focused on macroinvertebrate assemblages in debris dam habitats, and the findings will be relevant only to this particular habitat type. It is likely that the effects of flow permanence on macroinvertebrate assemblages in sandy benthic habitats will be greater than those in debris dams because these sections of the stream are the first to dry out when flow ceases and have little organic matter that can retain moisture. Debris dams also have long been considered to provide important habitat for macroinvertebrates and are the dominant source of energy in heavily forested streams (Bilby and Likens 1980; Smock et al. 1989).

Macroinvertebrates were sampled from debris dam habitats twice, once during the austral summer low-flow period (January 2007) and again during the spring high-flow period (November 2008). During the high-flow period, all sites had connected surface flow. During the dry period, the ephemeral stream had no surface water present and the streambed was dry although damp just below the surface. The intermittent stream had very little surface water present, although the streambed was slightly damp. The perennial stream had connected surface flow.

Ten debris dams were selected randomly from a 30 m reach in each stream and sampled by collecting one small handful of organic matter from the centre of the debris dam and preserving all material in 70% ethanol. Although these samples were not strictly quantitative, the use of this sampling technique in the catchment for other related investigations revealed no relationship between taxa richness and ash-free dry weight (AFDW, grams) of organic material collected in one handful ($R^2 = 0.10$ during 2007 and $R^2 = 0.03$ during 2008), despite some inevitable variation in the amount of organic material collected in one handful (4.71–32.47 g AFDW in 2007 and 3.82–31.98 g AFDW in 2008). Samples were washed over a 300 μm sieve in the laboratory, and insects were identified to the lowest possible taxonomic resolution (usually genus). Diptera were identified to family, except Chironomidae, which were identified to subfamily. Oligochacta and Nematoda were not identified further. Terrestrial taxa found in the samples were not included in any of the analyses presented here.

Data analysis

We plotted mean taxon richness and mean abundance for the three streams during both dry and wet periods. The distribution of abundance (evenness) among taxa was graphically explored using rank-abundance plots in which one axis of the curve represents the taxon rank in a community and the other represents taxon abundance (Tokeshi 1990), which was plotted on a log scale.

Given that we had three reaches of differing permanence over two flow seasons, we used a regression approach to analyze the response variables. The model was

$$(1) \qquad L(\mu_{a(j)}) = \alpha_1 + \alpha_2\delta_i + (\alpha_3 + \alpha_4\delta_i)P_j + b_j$$

where δ_i equals 0 for dry and 1 for wet. $L(\,)$ is a link function appropriate for measured values, $Y_{a(j)}$, where the subscript i denotes season (0 = dry, 1 = wet), j is reach (1, . . ., 3), and k is "replicate" debris dam within reach ($N = 10$). Note that the k subscripts are nested within the corresponding reaches j (hence $k(j)$). α_1 is the general intercept; α_2 is the effect of season, which is measured as a deviation from the dry season occurring in the wet season (hence the δ); α_3 is the regression parameter relating the response variable to the measure of permanence (F_j) in the dry season; and α_4 is an interaction that modifies the response–permanence relationship for the wet season. F_j is a continuous variable and was not selected a priori as a "factor." The bs are reach-specific random effects having zero mean and common variance σ_b^2 (these are used because of the "repeated measures" for each reach).

Different generalized linear models were developed to analyze patterns of richness (L = Poisson, deemed appropriate for nonnegative counts data), total abundance (L = lognormal scale), and evenness (L = Beta distribution) in the three reaches. Evenness was calculated using the preferred (Jost 2007) Shannon's equitability ($E(H)$), where $E(H)$ has a value between 0 and 1, with 1 indicating complete evenness (i.e., all taxa having equal abundances). Adequacy of model fit was assessed using Bayesian posterior predictive assessment (Gelman et al. 1996), which has been regarded as "Bayesian P values." "Importance" of the parameters (i.e., do these differ substantially from zero?) was assessed using posterior probability distributions and odds ratios (ORs). ORs are ratios of posterior to prior odds and measure how much the data change our initial expectations. ORs > 10 are strong

evidence supporting one hypothesis over another, and $3 < OR < 10$ signals positive evidence (Kass and Raftery 1995). For negative parameters, inverse ORs hold (i.e., ORs < 1/10 are strong evidence). Inferences in Results use OR ≥ 10 (or ≤ 1/10) as the threshold.

WinBUGS Bayesian modeling software was used (Spiegelhalter et al. 2003). Model runs had "burns-in" of 10 000 and parameter samples of 10 000. Usual appraisals of model convergence were employed (Smith 2006). More complete specifications of the Bayesian modelling are presented in the online Supplementary materials.[2]

Multivariate analysis exploring changes in community composition was undertaken using nonmetric multidimensional scaling (NMDS) for untransformed taxon abundance data. The ordination solution was generated from 100 random starts and is displayed in two dimensions due to low stress for this dimensionality (<0.15). The use of multivariate analysis of variance (MANOVA) or analysis of similarity (ANOSIM) to assess differences between groups was not undertaken because of the lack of true replicates (i.e., samples were subsamples at the reach scale). Centroids and 90% confidence ellipses were plotted for each group of 10 subsamples (R, version 2.4.1, http://www.r-project.org/). Effects of permanence on community composition were determined by visual inspection of the ordination plot.

Results

Regional drying and drought history (1961–2008)

The current period of drying in the region (2001–2008) was the longest period of consecutive years that streamflow has been below average since 1961 (Fig. 1). Although the cumulative streamflow deviation was large (in a negative direction), the deviation from any single year in the period 2001–2008 was not substantially greater than other negative annual streamflow deviations that have occurred in the past half century. In particular, the annual streamflow deviation in 1982 was very large (i.e., far below average) and annual streamflow deviations in 1962, 1967, and 1980 were also large.

Aquatic macroinvertebrate diversity in temporary vs. permanent streams

Seventy-four aquatic taxa (21 557 individuals) were found in the three headwater streams over the two sampling periods. Diptera was the most diverse order, with 11 families (Athericidae, Empididae, Ceratopogonidae, Chironomidae, Culicidae, Dixidae, Tipulidae, Psychodidae, Stratiomyidae, Simuliidae, and the rare Tanyderidae). Ephemeroptera, Plecoptera, and Trichoptera (EPT) collectively made up 35% of the total number of taxa recorded: 10 of the 25 freshwater Trichoptera families known to occur in Australia (13 genera), all four Plecoptera families known to occur in Australia (eight genera), but only two of the seven Ephemeroptera families (five genera).

Taxonomic richness

Using the OR criterion, all parameters differed from 0 with high certainty (all ORs infinite) (Table 1). Mean random effects for reaches (b_j) differed little from 0 when compared with their standard deviations (SDs) of estimates (all <10%). Model results are consistent with the data presented in Fig. 2*a*. Dry-season richness increased substantially with degree of permanence (from a mean of 4.9 (F = 0.08) to 26.2 (F = 0.83)). Wet-season richness was little related to the stream's permanence (Fig. 2*a*), which is consistent with the interaction effect for wet season as a function of permanence, which essentially eliminated the permanence relationship ($\alpha_3 + \alpha_4 = 2.28 + -2.29 = -0.01$).

Pooling all samples for each stream over the two sampling times revealed increasing total richness with degree of stream permanence: 42 (F = 0.08), 54 (F = 0.58), and 63 (F = 0.83).

Total abundance of aquatic macroinvertebrates

All parameters differed substantially from 0 (all ORs > 16; Table 1). Stream permanence had a marked positive effect on numbers of individuals in the dry season, increasing on average from about 12 individuals (P = 0.08) to 454 (P = 0.83) (Fig. 2*b*). Permanence depressed numbers for the wet-season data given a net difference of $\alpha_3 + \alpha_4 = (4.8–5.9) = -1.1$ in the model parameters. Thus, the model supports the evident interaction in which the wet-season total abundance decreased with flow permanence from an average of about 663 individuals (P = 0.08) to 347 (P = 0.83) (Fig. 2*b*). Mean random effects for reaches again were small, differing little from 0 when compared with their SDs of estimate and also absolutely (<0.09).

Rank-abundance plots showed that during the dry period, evenness was not markedly different among the three streams, although the plots appear quite different because of the reduced abundance and taxon richness in the two more-temporary streams (Fig. 3*a*). During the wet period, the slope of the three plots was very similar, indicating that evenness, abundance, and taxon richness were all similar among the three streams (Fig. 3*b*).

Evenness of abundances among taxa

All parameters differed greatly from 0 (all ORs > 32, Table 1). The baseline dry-season average evenness exclusive of permanence effects was 0.90. When the latter were included, fitted means (actual means in parentheses) for the permanent, intermittent, and ephemeral streams were 0.71 (0.66), 0.78 (0.79), and 0.89 (0.80), respectively. Thus, evenness decreased slightly with permanence for the dry season. The wet-season effect (exclusive of the permanence effect) reduced the baseline value to 0.47. The interaction term changed the regression coefficient for the permanence effect from −1.6 to +1.4 for the wet season. The fitted (actual) means for perennial, intermittent, and ephemeral streams were 0.74 (0.74), 0.67 (0.60), and 0.50 (0.57), respectively. Hence, evenness increased with permanence for the wet season. Mean random effects for reaches differed little from 0 when compared with their SDs of estimate. These results are supported by the rank abundance curves for each stream during the wet and dry periods, which graphically display patterns of evenness (Fig. 3).

[2] Supplementary materials for this article are available on the journal Web site (http:/cjfas.nrc.ca).

Fig. 1. Regional drying and drought history in the region represented by cumulative annual streamflow deviation for the period 1961–2008.

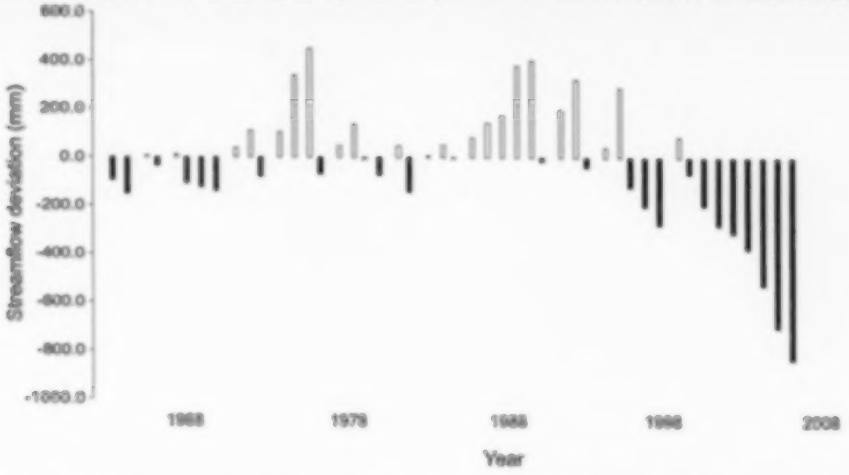


Table 1. Model parameter estimates (mean ± standard deviation, SD) for the analyses of species richness, abundance, and evenness.

Variable	Link (error)	α_1	α_2	α_3	α_4	PPP*
Richness	Log_e (Poisson)	1.4±0.3	1.8±0.2	2.4±0.6	−2.29±0.2	0.88
Abundance	Log_e (lognormal)	1.9±0.5	4.7±0.3	4.8±1.0	−5.9±0.5	0.78
Evenness	Logit (Beta)	2.2±0.5	−2.3±0.3	−1.6±1.0	3.0±0.4	0.69

*PPP, posterior predictive probability; values < 0.05 or > 0.95 suggest a poor model fit to the data.

Fig. 2. (*a*) Mean taxon richness and (*b*) mean abundance, with standard error bars in the three headwater streams during wet (solid diamonds) and dry (open diamonds) periods.

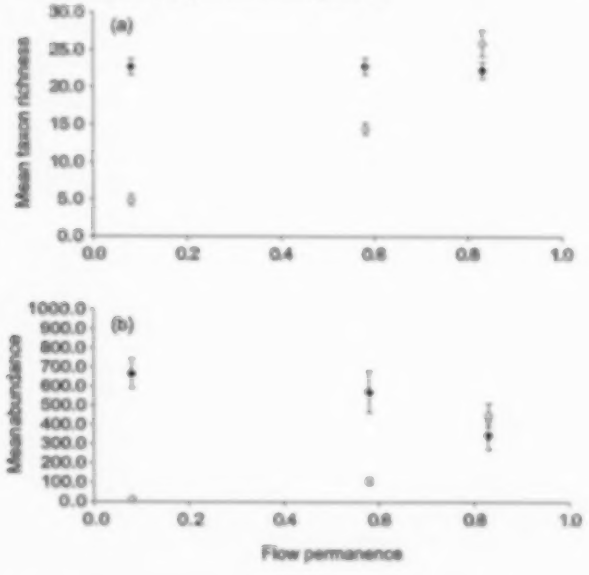
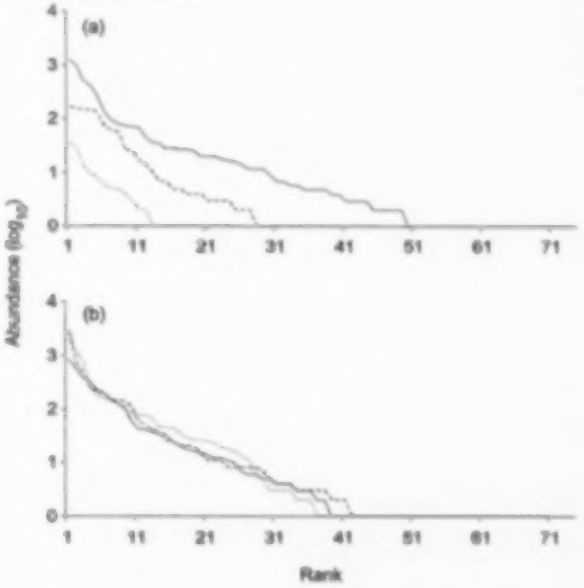


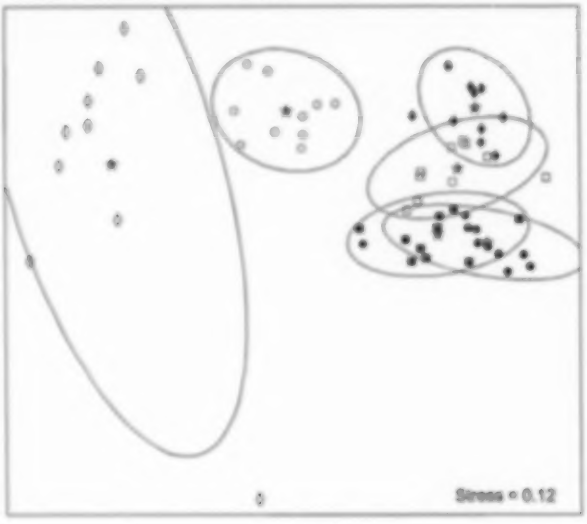
Fig. 3. Rank abundance curves (log-transformed) for (*a*) dry and (*b*) wet periods: solid line, perennial; broken line, intermittent; dotted line, ephemeral.



Aquatic macroinvertebrate assemblage structure in temporary vs. permanent streams

In the dry period, each stream had a distinct macroinvertebrate assemblage occupying debris dams with a clear grouping of samples separated by the difference in flow permanence for each stream (Fig. 4). The taxon list for each stream showed that during the dry period, the two temporary

Fig. 4. Nonmetric multidimensional scaling (NMDS) ordination of 60 samples from the three headwater streams during wet (solid symbols) and the dry (open symbols) periods: squares, perennial; circles, intermittent; diamonds, ephemeral.



streams had 12 taxa that did not occur in the perennial stream. The other taxa present in the two temporary streams were a nested subset of the taxa present in the perennial stream at the same time.

However, during the wet period, macroinvertebrate assemblages from each stream were very similar. Therefore, there is limited evidence from this study to support the idea that temporary streams in our region permanently harbour unique or distinctive macroinvertebrate communities associated with debris dams. Ordination based on presence–absence data revealed similar patterns (not shown).

Taxonomic responses to flow permanence in headwater streams

The Orthocladiinae (Diptera) showed the weakest response to flow permanence because this taxon was the most widely distributed across all streams and both sampling periods, absent from only three samples in the ephemeral stream during the dry period. The EPT group showed a very strong negative response to drying stress because sampling during the dry period in the intermittent stream found no taxa from the Plecoptera or Ephemeroptera and only three genera from the Trichoptera. Sampling in the ephemeral stream during this period revealed the absence of any genera from the EPT group. However, subsequent sampling of these streams during the wet period showed that eight genera of Plecoptera, three genera of Ephemeroptera, and four other genera of Trichoptera had colonized the intermittent stream, although one Trichopteran genus (*Lectrides* sp.) that had been present during the dry period was not recorded during the wet period. In the ephemeral stream, sampling during the wet period revealed that one genus of Plecoptera, two genera of Ephemeroptera, and two genera of Trichoptera had colonized the previously dry stream.

Of the 12 taxa found exclusively in the two temporary streams during the dry period, five were coleopterans (*Notohydrus* sp. (Hydrophilidae), *Hydraena* sp. (Hydraenidae), *Sclerocyphon* sp. (Psephenidae), Hydrochidae, and Curculionidae). Two dipteran taxa that appeared to be exclusively associated with driest conditions were in the families Ceratopogonidae (Forcipomyiinae) and Stratiomyidae as these were only recorded in the temporary streams and only during the dry period.

Discussion

Regional drying and drought history (1961–2008)

The streamflow deviation data indicate that the current drought represents the longest period of consecutive years of below-average streamflow in the study region since at least 1961, which is consistent with the widely reported descriptions of the prolonged nature of the drought that is currently affecting southeastern Australia (Pezza et al. 2008; Rose et al. 2008; McGowan et al. 2009). However, the occurrence of large (negative) annual streamflow deviations in earlier parts of the record (e.g., 1962, 1967, 1980, and 1982) means that macroinvertebrate communities in the region have previously been exposed to the pressure of highly variable and well below average streamflow. We might infer that macroinvertebrates in these systems may have evolved and maintained adaptations (e.g., life history, morphological, and behavioral) to cope with this highly variable flow regime, which is punctuated by drying and drought. Thus, differences in patterns of macroinvertebrate diversity between permanent and more temporary streams are likely to reflect structuring of communities in response to long-term patterns in hydrologic variability rather than just short-term responses to the current supraseasonal drought. However, if the current period of drought continues, the cumulative streamflow deviation in the region may become so severe that the hydrologic regime of some headwater streams will shift so that previously perennial streams become ephemeral and so on with consequent effects on the biota.

Effect of flow permanence on aquatic macroinvertebrate diversity

Stream ecologists have predicted that macroinvertebrate diversity should be low under conditions of intermittency because loss of hydrologic connectivity constrains exchanges of matter, energy, and organisms between patches (Poff and Ward 1989; Ward et al. 1999). This prediction may be less applicable to regions with naturally high levels of stream intermittency because periods of seasonal drying occur with a high level of predictability, allowing for development of adaptations to cope with the seasonal reduction or absence of flow. Several studies of macroinvertebrate responses to predictable, seasonal drying in Mediterranean-climate streams in regions such as California and Spain have found that intermittent and ephemeral streams do not always harbour a lower macroinvertebrate diversity than their perennial counterparts (Bonada et al. 2006, 2007). A study in Oregon found that a temporary headwater contained 125 species compared with only 100 species in a nearby permanent headwater (Dieterich and Anderson 2000).

In this study, we investigated the effects of flow permanence on macroinvertebrate assemblages in debris dam habitats and found that summer taxon richness was lower in the two temporary streams than in the permanent stream, but taxon richness in the intermittent stream was much higher than in the ephemeral stream. Taxon richness was similar in all streams during the wet period, which suggests that although many taxa might be seasonally extirpated or move from the channel into nearby refugia, they are extremely effective at recolonizing temporary streams upon resumption of flow.

The permanent stream had higher total (pooling sampling periods) taxon richness than either of the temporary streams, indicating that during the dry period, taxa are lost from the temporary streams but very few "new" taxa more tolerant of the dry conditions arrive to take their place. The ephemeral stream, which experienced the longest period without connected surface flow, had much lower taxon richness than the intermittent stream, and so annual taxon richness appears to be positively correlated with flow permanence. Similarly, Dieterich and Anderson (2000) found that although temporary streams may harbour as many or more taxa than nearby permanent streams, species richness was lowest at the most ephemeral sites. Boulton and Lake (1992) compared macroinvertebrate assemblages between sites on two intermittent rivers in southern Australia and also found that species richness generally increased with longer flow duration.

Evenness was relatively high in all three streams, even during the dry period when the temporary streams contained few taxa. This pattern was driven by many taxa in the dry period occurring only as one or two individuals and no single taxon occurred in exceptionally high abundances. This result is consistent with a study of headwater streams by Heino et al. (2008), who found high evenness in small streams and inferred that this phenomenon might reflect so called "small stream effects" where abundances are more evenly divided among species because of inherent heterogeneity and variability in the conditions of small headwater streams.

Effect of flow permanence on macroinvertebrate assemblage structure

Towns (1985) suggested that fauna inhabiting temporary waters are highly specialized with unique life history adaptations to intermittent flow. However, several studies have reported only slight differences in the faunal composition of permanent and intermittent sites (Boulton and Lake 1992; Feminella 1996; Price et al. 2003). We found that although there were seasonal differences in assemblage structure, patterns were mostly driven by loss of taxa and there was a high degree of faunal overlap between the three sites once flow resumed. Only 12 taxa (16%) were found exclusively in the temporary streams. These findings suggest that the temporary streams in our study region do not contain a significant suite of specialist taxon adapted to intermittent flow and that for the many taxa that are seasonally extirpated from the temporary streams, recolonization from populations in the permanent stream is likely to occur in the wetter months.

Taxon responses to flow permanence in headwater streams

Orthocladiinae appeared to be the best adapted to a range of flow permanence, which is not surprising as they are known to occupy the widest range of habitats of all the Chironomidae (Oliver 1971). A substantial number of species from the Orthocladiinae are multivoltine with continuous recruitment for much of the year (Pinder 1986).

Of the EPT group, the Trichoptera appeared to have the greatest ability to withstand or recover from drying stress in our study, although their ability to survive in temporary streams during the dry period seemed limited to genera known to build a case or retreat of some type (*Caenota* sp., *Lectrides* sp. and genus Calocidae B). Larval case building by caddisfly taxa has previously been suggested as a form of resistance to drying or drought (Zamora-Munoz and Svensson 1996). Although eight genera of stoneflies colonized the intermittent stream once flow had resumed, only one genus was found to have colonized the ephemeral stream during the wet period, *Dinotoperla* sp. (see also Boulton and Lake (1992)).

Most aquatic Coleoptera and the Tipulidae (Diptera) pupate out of the stream channel in cells formed by damp soil and moss, a habitat that is prevalent when flow recedes or ceases (Cummins and Wilzbach 2005). This may explain the prevalence of coleopteran and dipteran taxon found in the temporary streams in our study (Boulton and Lake 1992). Price et al. (2003) found that intermittent and ephemeral streams tended to be richer in dipteran taxon, and Bêche et al. (2006) found that during the dry season, density of air-breathing macroinvertebrates with strong body armouring such as the Coleoptera increased. The success of dipteran taxa in colonizing temporary habitats is likely to be due to a suite of functional and life history traits that prove advantageous such as short life cycles, simple bodies, survival in a wide range of habitats, and high dispersal ability of adult stages (Oliver 1971; Pinder 1986).

Implications for management of intermittent headwater stream systems

Periods of drying and drought are an integral part of the Australian climate (Rose et al. 2008), and indigenous macroinvertebrate communities from such Mediterranean-type climates are likely to be adapted to naturally occurring low-flow or zero-flow conditions (Boulton 2003; Lake 2003; McMahon and Finlayson 2003) or have highly efficient mechanisms for recolonization when flow resumes, as suggested by the findings of our study. However, taxa may not be as resilient or resistant to the interactive effects of predictable seasonal drying and longer-term supraseasonal drought (Lake 2003) such as the current severe and prolonged drought occurring in southeastern Australia (Rose et al. 2008; McGowan et al. 2009). Analysis of long-term rainfall records in southeastern Australia (1910–2005) showed a decline in most rainfall indices, particularly since the 1950s when total autumnal rainfall began decreasing by approximately 11 mm per decade, contributing to a significant decrease in total annual rainfall of approximately 20 mm per decade (Gallant et al. 2007). Further intensifying the effects of natural intermittency of some headwater streams are increasing levels of water extraction from unregulated rivers and the future effects of climate change that are predicted to be substantial for upland streams (Durance and Ormerod 2007).

Managing freshwater systems in the face of growing demand for water resources and the predicted impacts of climate change will be challenging, but it is critical that actions to protect freshwater biodiversity be taken (Dudgeon et al. 2005). Although intermittent streams may harbour a lower diversity than their perennial counterparts during some parts of the year, it appears from our study that intermittent headwater streams may regain their full complement of taxa upon resumption of flow. However, these findings are limited to macroinvertebrate assemblages in debris dam habitats, and the effect of flow permanence on macroinvertebrate assemblages in sandy, benthic areas may be more pronounced due to the rapid onset of drying in these sections of stream once flow ceases. The permanent stream in our study may have provided a critical oversummering refuge for many taxa; Boulton (1989) highlighted the importance of such streams as sources of recolonization. In this system, taxa from the perennial stream appear extremely efficient at colonizing the seasonally dry, temporary streams. When planning for reserve design or disturbance mitigation, it may be important to ensure that intermittent headwater streams are not disconnected from nearby permanent streams by blocking or fragmenting aerial and upstream migration pathways. This study also highlighted the potential loss of diversity, at least seasonally, that may occur as the local and regional climates become drier and previously perennial and intermittent streams shift towards more ephemeral flow regimes.

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Assessing habitat utilization and rockfish (*Sebastes* spp.) biomass on an isolated rocky ridge using acoustics and stereo image analysis

Christopher N. Rooper, Gerald R. Hoff, and Alex De Robertis

Abstract: For those marine fish species with specific habitat preferences, a habitat-based assessment may provide an alternative to traditional surveys. We conducted a habitat-based acoustic and stereo image stock assessment survey for rockfishes (*Sebastes* spp.) on a rocky ridge habitat in the eastern Bering Sea. Video analysis suggested that juvenile and adult rockfishes were more abundant on the seafloor in the rocky ridge area than on the surrounding sandy flats. Over the ridges, the distribution of rockfishes was uniformly low in the water column during nighttime surveys and higher during daytime surveys. The opposite pattern was observed in the video on the seafloor between night (high density) and day (lower density), indicating that fish in the water column during the day moved to the seafloor at night. Mean biomass of adult rockfishes for the rocky ridges was 1.54×10^4 tonnes based on acoustic data. The biomass of juvenile fish was estimated to be 9.2×10^2 tonnes. Utilization of similar survey methodologies on a larger scale might improve assessment of rockfishes not only in Alaska, but also throughout their range where fishery-independent biomass estimates have been difficult to obtain.

Résumé : Chez les espèces de poissons marins qui possèdent des préférences spécifiques d'habitat, une évaluation basée sur l'habitat pourrait être une méthode de rechange aux inventaires traditionnels. Nous avons fait un inventaire acoustique et vidéo d'évaluation des stocks des sébastes (*Sebastes* spp.) basé sur l'habitat dans un milieu de crête rocheuse dans l'est de la mer de Béring. L'analyse vidéo indique que les sébastes jeunes et adultes sont plus abondants sur le fond marin dans la région de la crête rocheuse que sur les plats sablonneux adjacents. Sur les crêtes, la répartition des sébastes est uniformément basse dans la colonne d'eau durant les inventaires de nuit et plus élevée durant les inventaires de jour. Un patron contraire s'observe par vidéo sur le fond marin entre les inventaires de nuit (densité élevée) et de jour (densité basse), ce qui indique que les poissons qui sont dans la colonne d'eau durant le jour se déplacent vers le fond marin la nuit. La biomasse moyenne des sébastes adultes sur les crêtes rocheuses est de 1.54×10^4 tonnes d'après les données acoustiques. La biomasse des jeunes poissons est estimée à 9.2×10^2 tonnes. L'utilisation de méthodes semblables d'inventaire sur une plus grande échelle pourrait améliorer l'évaluation des sébastes, non seulement en Alaska, mais aussi sur toute leur aire de répartition dans laquelle des estimations des biomasses indépendantes de la pêche sont difficiles à obtenir.

[Traduit par la Rédaction]

Introduction

In addition to fishery-dependent data, fishery-independent surveys of marine fish species have been a primary data source for producing fisheries stock assessments for commercially exploited species. A serious problem in estimating fish biomass occurs in multispecies surveys where catchability or availability of the fish to the survey may change with habitat type (Cordue 2007). This is especially true of bottom trawl surveys where all habitat types may not be sampled equally with the survey gear (Zimmermann 2003). Additionally, recent concern with essential fish habitat requirements for many species, as well as the potential for damage of habitat by both commercial and research fishing gear (Van Dolah et al. 1987; Freese et al. 1999; Krieger 2001), suggest alternative approaches may be needed for some species of fish.

One solution may be in habitat-based assessment surveys for some fish species that have clear habitat affinities. Generally, habitat-based assessment surveys produce fishery-independent estimates of biomass by applying the mean density within a habitat to the total area that the habitat comprises in the ecosystem. Habitat-based assessments have been used for a number of species where other methods of fishery-independent surveys are not possible, e.g., cowcod (*Sebastes levis*) and yelloweye rockfish (*Sebastes ruberrimus*). In the case of cowcod, the small population size

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necessitates use of nonextractive survey gear (Yoklavich et al. 2007), whereas in the case of yelloweye rockfish, their affinity for rough areas makes a bottom trawl survey impossible (O'Connell and Carlile 1993).

Estimating the biomass or abundance of northern rockfish (*Sebastes polyspinis*) and Pacific ocean perch (*Sebastes alutus*) in Alaska and on the US West Coast is challenging due to their semipelagic schooling behavior and affinities for rocky untrawlable habitat (Clausen and Heifetz 2002; Rooper et al. 2007). Both of these characteristics make their abundance difficult to assess using bottom trawl survey methodology. There is evidence that species assemblages differ among habitats and that untrawlable areas can have different species than trawlable areas (Matthews and Richards 1991; Jagielo et al. 2003; Zimmermann 2003). Untrawlable areas can also have different size classes or abundances of the same species (Matthews 1989; Stein et al. 1992; Rooper et al. 2007). These patterns in habitat use can have a significant effect on the accuracy and precision of bottom trawl survey biomass estimates for some species of rockfishes (Cordue 2007). Resource assessments for these species are commonly conducted with stratified random bottom trawl surveys in which catch-per-unit-effort (CPUE) data for each species is typically expanded across entire strata to estimate the total biomass for a species (e.g., Wakabayashi et al. 1985). When CPUE from a randomly stratified bottom trawl survey is applied across the entire area regardless of the habitat preferences of the species or the trawlability of the grounds contained therein, biased biomass estimates may result. Thus, there is a serious need for habitat-specific assessment methods for rockfish species in regions that include untrawlable areas.

Use of acoustics to measure the biomass of fishes has long been a staple for pelagic fishery-independent assessments. Several recent studies have demonstrated the feasibility of acoustical techniques to assess rockfish biomass in the North Pacific (Wilkins 1986; Stanley et al. 2000; Ressler et al. 2009). These studies have estimated the biomass of midwater rockfishes, which are more accessible to the acoustic instruments than those very close to the bottom, particularly in high-relief terrain (e.g., Ona and Mitson 1996; Cooke et al. 2003).

One issue that arises with acoustical methods is estimation of the size distribution and species composition of the acoustically observed targets (McClatchie et al. 2000). In midwater assessments such as those done for walleye pollock (*Theragra chalcogramma*), a series of midwater trawl hauls are conducted in conjunction with the acoustic survey to confirm the size and species composition of acoustic targets (e.g., Karp and Walters 1994). Stereo image analysis provides an alternative to trawl surveys in areas where trawls cannot be used and where species composition is relatively easy to ascertain from underwater cameras. Cameras can also detect fish in the near-bottom echosounder dead zone that is inaccessible to the acoustic equipment (Ona and Mitson 1996). The combination of acoustic technology with stereo optics can provide a nonextractive method for assessment of rockfishes in untrawlable areas (Demer et al. 2009).

Our objectives were (*i*) to describe substrate preferences of rockfish species, (*ii*) to examine day–night differences in habitat use and abundance by rockfishes, and (*iii*) to estimate the biomass of rockfishes and species composition using a combination of acoustics and underwater video observations on the Zhemchug ridges.

Materials and methods

This study was conducted at the Zhemchug ridges, which are two isolated rocky ridge areas located on the eastern Bering Sea shelf adjacent to Zhemchug Canyon (Fig. 1). The eastern Bering Sea shelf is notable for its large production of flatfish, gadoid, and crab species and their associated commercial fisheries. Rockfishes occur in large numbers on the eastern Bering Sea continental slope but are common on the shelf only in areas of rocky relief (Hoff and Britt 2005). The Zhemchug ridges comprise one of the only known rocky outcroppings on the eastern Bering Sea shelf. These ridges rise sharply from the flat sandy seafloor (125 m) to depths of <85 m. For this study, two habitats were defined: the "ridge" areas, habitats defined by the sharply narrowing depth contours from the seafloor to the ridge tops (Fig. 1), and the "flats", surrounding areas where depth varies little. The ridges are located at the southern horn of Zhemchug Canyon, between the Bering Sea continental shelf and the upper canyon wall, both of which drop steeply to depths > 2000 m. This study was conducted at Zhemchug ridges over four days from 12 to 15 July 2008.

We first determined the distribution of habitats and different seafloor substrates in the Zhemchug ridges area using underwater video imagery. We then measured the acoustic backscatter from fish in the water column during daytime and nighttime surveys to investigate changes in their diel distribution, and we identified species and size composition of fish observed acoustically in the water column using underwater stereo video during paired daytime and nighttime dives. Finally, we used the combined analysis of underwater video and acoustic backscatter to determine rockfish habitat use by size and species and to estimate rockfish biomass.

Underwater video data collection and analysis

Underwater video was collected using a drop camera sled (Williams et al. 2010) constructed of aluminum tubing to protect camera and lighting components. Two lights (each 50 W high-intensity discharge, HID) powered by 12 V NiMH battery packs provided illumination of the seafloor. Video was collected by two battery-powered Sony TRD-900 progressive scan camcorders mounted in housings on the sled. The port inside each housing was keyed so that the position of each camera relative to the other was kept constant. These cameras were forward-facing and mounted side by side in parallel to provide stereo video capabilities. The camera sled drifted through the water column approximately 1–2 m above the substrate pointed slightly downward at an angle of approximately 30° off parallel to the seafloor. The cameras collected up to 1 h of color video at 720 p (720 horizontal lines of progressive scan video resolution). One of the cameras was connected to the surface through a 400 m electromechanical cable and winch with a slip ring to provide real-time video that assisted in navigation over the uneven seafloor.

Fig. 1. Study site at Zhemchug ridges showing transect lines for the acoustic survey (black lines), as well as sites where the underwater stereo video camera was deployed (solid circles indicate transects conducted at night; open circles are daytime transects). The thick shaded line indicates the areal extent of the two ridges and the medium shaded line indicates the extent of the surrounding flats. Thin shaded lines indicate bathymetry in 10 m intervals (from 90 m to 150 m) inside the study area and in 100 m intervals from NOAA charts beginning with 200 m outside the study area. The box in the inset shows the relative location of the Zhemchug ridges on the eastern Bering Sea slope.

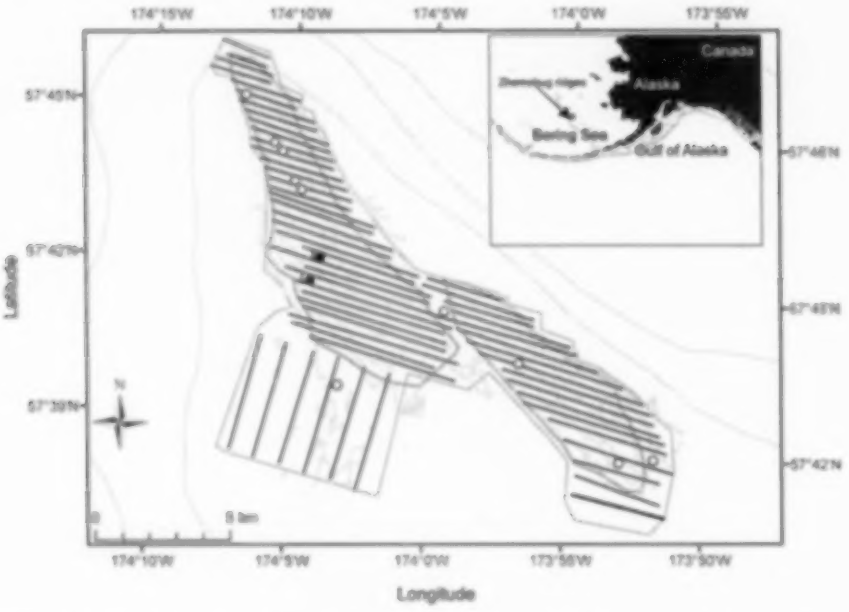


Table 1. Summary of data by habitat type and daytime or nighttime, including the number of each primary species observed in the video, the number of transects in each habitat, and the total area (ha) of seafloor observed in the video.

	Flats	Ridge	Day (ridge)	Night (ridge)
Number of transects	3	7	2	2
Total area observed (ha)	0.54	1.53	0.53	0.49
Rockfishes				
Juvenile Pacific ocean perch (*Sebastes alutus*)	81	2271	459	1232
Adult Pacific ocean perch	0	1	1	47
Northern rockfish (*Sebastes polyspinis*)	0	1305	8	2763
Dusky rockfish (*Sebastes variabilis*)	0	25	10	20
Unidentified	0	6	4	9
Other fishes				
Atka mackerel (*Pleurogrammus monopterygius*)	0	39	0	3
Yellow Irish lord (*Hemilepidotus jordani*)	0	56	0	2
Pacific cod (*Gadus macrocephalus*)	1	23	23	1
Searcher (*Bathymaster signatus*)	50	471	177	16
Pacific halibut (*Hippoglossus stenolepis*)	2	1	2	0
Unidentified flatfish	5	4	0	0
Unidentified fish	10	41	3	11

Video was collected over 14 transects ranging in duration from 3.5 to 49.5 min of on-bottom video collection and covering distances of 95 to 1673 m. Time and position data were collected from the vessel global positioning system during each underwater video transect. The on-bottom time and location were recorded when the seafloor was first sighted in the video. The off-bottom time and location were recorded at the end of each transect when the camera sled left the seafloor. The camera was successfully deployed during daytime at 10 transects randomly selected to represent both the north and south ridges ($n = 7$) and the flat areas surrounding the ridges ($n = 3$) (Table 1). In addition, two transects were sampled during nighttime ($n = 2$) and the following daytime ($n = 2$) over the north ridge habitat to provide day–night comparisons.

Substrate classification

The video from one of the two cameras was analyzed for each transect to determine substrate classification according to the method of Stein et al. (1992) and Yoklavich et al. (2000). In this method, the substrate classification scheme consists of a two-letter coding of substrate type denoting a primary substrate (>50% coverage of the seafloor) and a secondary substrate (20%–49% coverage of the seafloor). There were six substrate types observed at our study site: sand (S), gravel (G, diameter < 6.5 cm), cobble (C, 6.5 < diameter < 25.5 cm), mixed coarse material (MC, an almost even mix of gravel and cobble), boulder (B, diameter > 25.5 cm), and bedrock (R). The approximate size of substrate components was estimated from the video by the observer. For example, a section of seafloor covered primarily in sand but with boulder-sized rocks comprising more than 20% of the surface would receive the substrate code sand–boulder (Sb), with the secondary substrate indicated by the lower-case letter. Similarly, a patch of seafloor consisting almost entirely of bedrock (>80%) with no secondary substrate would be coded Rr. Because the underwater video sled provided a continuous display of substrates, the substrate coding was only changed if a substrate encompassed more than 10 consecutive seconds of video (Tissot et al. 2006). The video analysis was carried out by a single observer who recorded the substrate classification.

Fish identification

Rockfishes in each video were identified to species by a single observer, and each fish was initially classified as an adult (>250 mm) or juvenile (<250 mm) based on its approximate size. Two decision rules were used to determine if a fish was counted; if the eye of the fish was observed in the video screen and if the fish was observed in the lower 75% of the video screen. Given these conditions, all fish were assumed to be detectable. As has been observed in other studies of rockfishes near the seafloor (Else et al. 2002; Nasby-Lucas et al. 2002; Lauth et al. 2004), there was little indication of a response to the camera sled during either the day or the night. In general, rockfishes either maintained a steady position as the sled passed or moved out of the direct path of the sled as it approached at distances < 1 m. Escape responses, where a fish darted away from the path of the sled, were rarely observed except under abnormal conditions such as when the camera sled struck a rock. The same videos were used for fish identification and enumeration as were used for substrate classification. In this study, the rockfishes were identified into five species groups: adult Pacific ocean perch, adult dusky rockfish (*Sebastes variabilis*), adult northern rockfish, unidentified adult rockfishes, and juvenile rockfishes. The substrate type where the fish occurred was also noted for each individual. Each video transect was viewed in its entirety three times by the same observer, with individual sections viewed multiple times when necessary to accurately enumerate and identify the fish and substrate type. Fish density from video observations was estimated for each transect as the number of fish divided by the area observed in the video. The area observed in the video was the distance traveled over the seafloor multiplied by the average video path width (defined as the average width of the viewable seafloor along each transect).

Fish length measurement

All fish lengths and video path widths were measured by standard stereo image analysis techniques (Bouguet 2008). Because the stereo image analysis is carried out on still-frame images, each video was subsampled for paired (left and right camera) still frames at 1 s intervals. The images were synchronized at the beginning of each transect prior to deployment using an LED (light-emitting diode) timing light that was recorded simultaneously by both cameras. The images were checked at the end of each transect to confirm that the cameras were synchronized.

The two cameras were calibrated at the beginning and end of the study using a checkerboard target of known dimensions. The stereo calibration parameters were estimated with a freely available analysis toolbox built using Matlab computing language (Bouguet 2008; The Mathworks Inc. 2008). During calibration, the position of corner points of the checkerboard were identified in multiple frames of the video (20 per camera), and the calibration software used the relative location of these points in the still images to solve for the internal optical parameters of each camera.

For stereo calibration, the corresponding checkerboard corner points in two corresponding images are used to compute the epipolar geometry. Knowing the internal camera parameters and the position of one camera relative to another, the position of a target point viewed in both cameras could be determined by triangulation. Once the internal camera parameters are estimated by the software, the three-dimensional position of a target point viewed in both cameras can be determined by triangulation.

Length measurements could be obtained by determining the positions of two identical points in each of the paired still frames (such as a fish's snout and tail observed in both still images). The calibration was tested by measuring the length of known distances on the checkerboard at the beginning and end of the study. A series of independent frames from both cameras of the target checkerboard were collected at these times. The difference in measurements between the beginning and end of the study was approximately 7.8% (standard error, SE = 0.1) (Williams et al. 2010). This was most likely due to slight misalignment of the cameras relative to their position during calibration that occurred when the cameras were removed from the housings to change the videotape after each deployment.

Still-frame images were available from both the left and right cameras in 11 of the 14 transects. Individuals of each species group were chosen at random to be measured according to their percentage of the total rockfishes observed. Up to 200 individual rockfish were measured in each transect. The randomly selected rockfishes were measured for fork length (centimetres). Rockfishes were only measured if both the tip of their snout and the end of the tail were plainly visible in both still images. If the randomly chosen rockfish was not measureable, the next available rockfish of the same species group that was deemed measureable was chosen. In a few cases, where occurrence of a species group was very small (< five individuals in a transect), none was measured. The video path width was measured from 20 randomly selected still-frame images from each transect. For each of these frames, the distance from the farthest left extent of seafloor that could be seen to the farthest right extent

was measured at a consistent level (one-half of the image height). The average video path width across all transects was 2.43 m (SE = 0.14).

The length–frequency distributions (weighted by density) for each species were compared among daytime video transects between the ridges and the flats. The length distributions were also determined for the paired daytime and nighttime transects on the north ridge. In the three transects where no lengths were available, the average length distribution was assigned. For example, at one of the nighttime transects on the north ridge, still images were not available from the left camera. In this case, the nighttime length distribution from the other transect was used.

The rockfishes that were observed on the seafloor only during the night were assumed to be in the water column during the day. Therefore, subtracting the daytime length distribution from the nighttime length distribution gave an estimate of the length distribution of fish in the water column during the day. Similarly, the differences in species composition on the seafloor during nighttime and daytime periods were also used to estimate the species composition of the fish in the water column during the day.

Acoustic survey data collection

Pelagic fish abundance was measured using a 38 kHz single-beam Simrad ES60 echosounder equipped with a hull-mounted 38-22 transducer (22° × 11° beam width). The echosounder was calibrated using the standard sphere method (Foote et al. 1987) prior to the beginning of the project (on 1 June) and at the end of the project (on 8 August). The calibration sphere was composed of pure copper and was 60 mm in diameter. The mean integration gain from these calibrations (averaged in linear units) and the nominal equivalent beam angle supplied by the manufacturer for this transducer model were used for subsequent data analysis.

The acoustic survey design consisted of a series of transects across the ridges and in the flat area to the south of the ridges with an intertransect distance of approximately 0.463 km (0.250 nautical miles, nmi) (Fig. 1). Three separate surveys were done in each area. The first survey was conducted in all three areas during daylight hours. A second survey was performed during nighttime hours over the same transects. Finally, another daytime survey was completed in all three areas but with the transect offset from the original daytime survey transects by 0.232 km (0.125 nmi). Transects were offset to obtain maximum coverage of the depth contours for each ridge area and to thereby produce the most accurate maps possible. At the north ridge, only half of the initial daytime survey was completed due to an equipment malfunction.

Acoustic data analysis

The ES60 echosounder has a periodic embedded error that results in a difference of up to ±1 dB (±23%) in acoustic measurements made on single transmissions (T. Ryan, CSIRO Marine and Atmospheric Research, Castray Esplanade, Hobart 7001, Australia, personal communication, 2004). This error was removed from all of the data used in this study using the method of Keith et al. (2005), which quantifies the error by analyzing the degree to which the constant transmit pulse in the first few acoustic samples is perturbed by the error and then corrects for the error. These postprocessed records were integrated to estimate the nautical area scattering coefficient (s_A, $m^2 \cdot nmi^{-2}$) from 2.5 m above the sounder-detected bottom to 40 m below the surface over horizontal transect segments of 200 m. A boundary of 2.5 m above the sounder-detected bottom was chosen because of both the rugose seafloor and the observation that the underwater video camera was able to estimate the near-bottom component of the rockfish population. The upper boundary of 40 m below the surface was chosen because it was above the rockfish backscatter in all cases and eliminated acoustic noise and small scatterers near the surface.

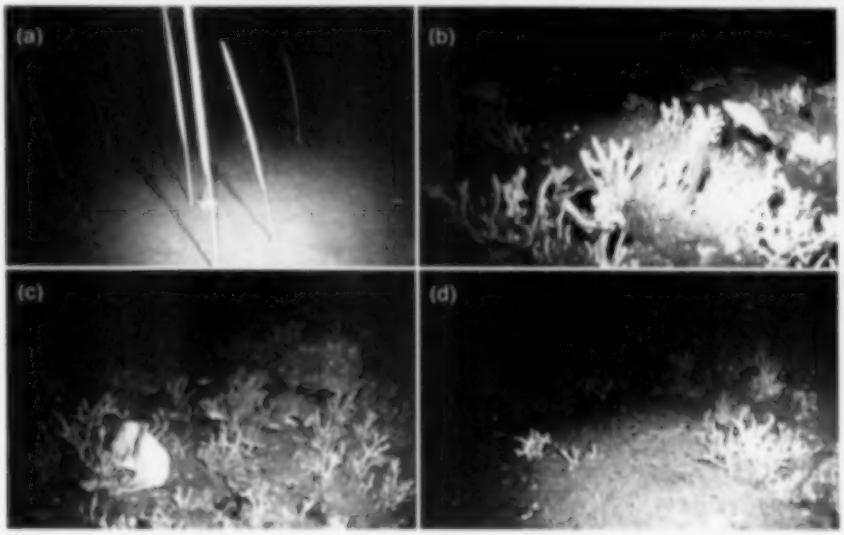
Biomass estimation

The fish biomass was estimated for each of the daytime surveys over the rocky ridge habitat. We were not able to estimate target strength (TS) of rockfishes in the area during this study, and there are no published estimates for the species encountered during the study. As a first-order approximation, we used $TS = 20 \log_{10} L - 67.5$, where L is length (in centimetres) (Stanley et al. 2000). This generic relationship for physoclist fishes was used in studies of widow rockfish (*Sebastes entomelas*) off British Columbia. A length distribution and species composition for rockfish species were derived from the stereo camera measurements obtained from video transects. The TS regression described above, the length–frequency distribution from the stereo camera, and s_A from the echosounder were used to estimate the numerical density of rockfishes in 1 cm length classes in the study area following Simmonds and Mac Lennan (2005). Because juvenile (predominantly Pacific ocean perch) and adult rockfishes (predominantly northern rockfish) comprised the vast majority of the fish observed in the video, separate weight–length relationships (juveniles, $W = 1.13 \times 10^{-5} L^{3.04}$ (Rooper 2008); adults, $3.26 \times 10^{-6} L^{3.24}$ (Hoff and Britt 2009)) were used to estimate the density in terms of fish weight from the density in terms of fish numbers. The density of fish weight was then converted to total biomass for each group by applying it to the total area surveyed (56.9 km^2). Simple confidence intervals for adult and juvenile biomass estimates were based on the variance of the two daytime surveys and calculated using $\alpha = 0.05$.

Data analysis and hypothesis testing

Habitat use by juvenile and adult rockfishes was compared to evaluate three hypotheses: (*i*) that juvenile and adult rockfishes are found in the same densities in ridge and flat habitats, (*ii*) that daytime and nighttime distributions were the same, and (*iii*) that juvenile and adult rockfishes were randomly distributed by substrate type. A t test for differences in fish density among habitats with $n = 10$ video transects was used to test for differences in habitat use for each species. Similarity, in daytime and nighttime, distributions of juvenile and adult rockfishes on the seafloor were tested with a t test of the data from the paired video transects during daytime and nighttime ($n = 4$). A χ^2 test was used to test whether rockfishes were randomly distributed by substrate type.

Fig. 2. Still images from the four major substrate types encountered during underwater video transects of the Zhemchug ridges. The substrates represented are (*a*) sand (Sa), (*b*) bedrock (Rr), (*c*) bedrock with boulders (Rb), and (*d*) sand with boulders (Sb).



Additionally, an analysis of variance (ANOVA) was used to compare the pelagic fish distribution observed in the flats and ridges during the daytime and nighttime. The ANOVA contained area (north ridge, south ridge, and flats), survey (daytime 1, daytime 2, and nighttime), and an interaction term as independent variables and s_A for each transect as the dependent variable (n = 91). Significance was determined at $p < 0.05$.

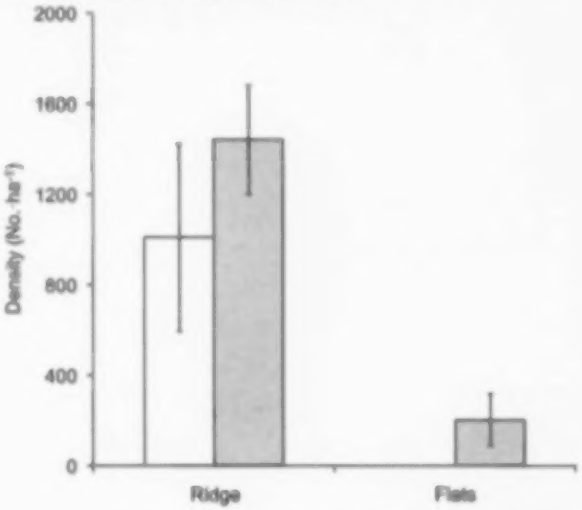
Results

The flats were dominated by sandy substrates (87.1%) with some sand–boulder substrate (5.8%) and bedrock with boulders (6.7%). The ridges were primarily composed of bedrock (44.2%) and bedrock with boulders (50.9%), although some sandy substrates (2.8%) and sand–boulder substrates (1.5%) were present as well. Most of the hard substrate (bedrock and boulders) had attached benthic invertebrates, and at two of the sand transects, there were significant numbers of seawhips and hydroids present (Fig. 2).

Juvenile and adult rockfishes were more abundant on the seafloor in the rocky ridge area than on the sandy flats (Fig. 3). Densities of juvenile rockfishes were significantly higher on the ridges than on the flats, and no adult rockfishes were observed on the flats. The adult rockfish species composition on the seafloor was dominated by northern rockfish (96.94%), while unidentified adult rockfishes (0.98%), adult Pacific ocean perch (0.49%), and dusky rockfish (1.60%) comprised only small portions of the adult rockfishes observed in the video. Most of the juveniles that were identified to species were Pacific ocean perch; however, many smaller specimens could not be positively identified, so they were lumped together as juvenile rockfishes.

Densities of juvenile and adult rockfishes on the seafloor were higher during the nighttime than during the daytime, although these differences were not statistically significant (Fig. 4). Adult rockfishes were roughly two orders of magnitude more dense on the seafloor during the night, whereas juvenile rockfishes were about twice as dense during the night versus the day. The lack of statistical significance was most likely because of the small sample size comparing night and day (n = 2).

Fig. 3. Densities and standard error bars of juvenile (shaded bars) and combined adult (open bars) rockfish species for flat and ridge areas of Zhemchug ridges habitat. Densities were averaged for observations of rockfish abundance at transects in ridge (n = 7) and flat (n = 3) habitats using underwater video.



The χ^2 test for goodness-of-fit indicated that neither juveniles nor adults were randomly distributed across substrate types ($p < 0.0001$ and $p < 0.0001$, respectively). Juvenile and adult rockfishes were found predominantly in boulder fields and rocky substrate (Table 2). Only 74 juveniles and nine adults were observed in sand substrate, although a sig-

Fig. 4. Daytime and nighttime densities and standard error bars for juvenile (shaded bars) and adult (open bars) rockfishes near the seafloor measured from paired daytime and nighttime video transects at the same locations ($n = 2$).

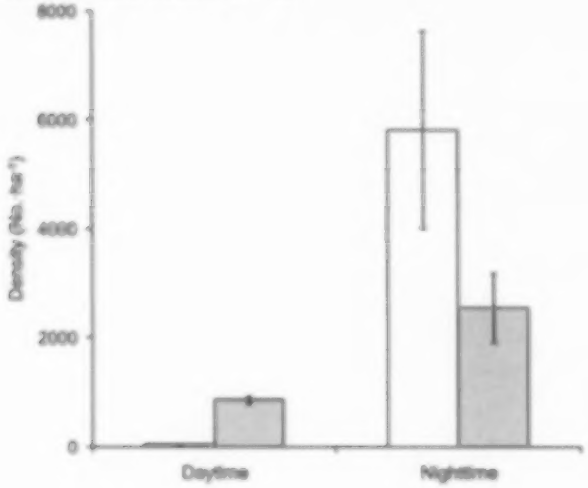


Table 2. Percentages of fish per substrate and substrates observed in underwater video transects at the Zhemchug ridges.

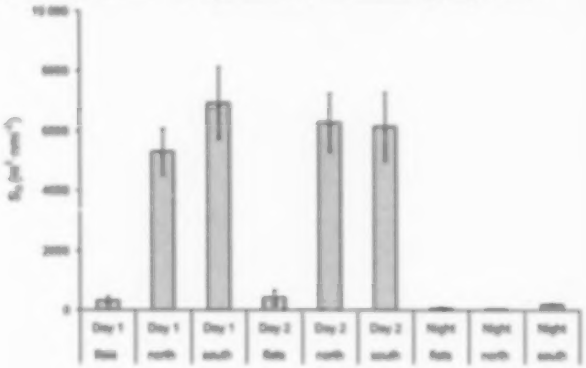
		Rockfishes observed (%)	
Substrate code	Total substrate (%)	Adults	Juveniles
Gg	0.23	1.64	0.25
MCc	0.11	0.00	0.00
MCmc	0.21	0.00	0.00
Rb	43.18	28.01	46.33
Rr	36.60	69.75	38.39
Sb	2.24	0.38	13.21
Ss	17.42	0.21	1.83

Note: Rockfishes, *Sebastes* spp. The two-letter coding of substrate type denotes a primary (upper-case letters used for primary substrate; >50% coverage of the seafloor bottom) and a secondary (lower-case letters used for secondary substrate; 20%–49% coverage of the seafloor) substrate: gravel, G; sand, S; mixed course material, MC; cobble, C; boulder, B; bedrock, R.

nificant portion of the juvenile rockfishes were observed in the mixed sand–boulder substrate. The adult rockfishes were almost exclusively found in the rocky or rocky–boulder substrates (>97%), even though this substrate comprised only about 79% of the substrate examined.

The ANOVA testing for differences in pelagic fish abundance (measured by the average s_A value) found that fish abundance in the water column was significantly higher over the ridge features than over the flats (Fig. 5). There were significantly more fish in the water column during the two daytime surveys than during the nighttime survey. There were no significant differences in the average s_A values between the north and south ridges or between the first and the second daytime surveys. The distribution of s_A values was uniformly low during the day over the flats and much higher over the ridges (Fig. 6).

Fig. 5. Mean nautical area scattering coefficients (s_A, $m^2 \cdot nmi^{-2}$) and standard error bars in the water column during day and night in the different habitat types (ridge and flats) for each of the three surveys (two daytime and one nighttime) of the Zhemchug ridges area.



The mean sizes and size distributions of adult rockfishes on the seafloor were similar for both night and day. Interestingly, the mean size of juvenile rockfishes was significantly smaller during the daytime (11.4 cm, SE = 0.39) than during the nighttime (18.8 cm, SE = 0.36) due to the absence of the smallest sizes (<14 cm) of juvenile rockfishes during the nighttime (Fig. 7). During the nighttime when extremely high numbers of adult rockfishes were present, the smallest juveniles may have sought cover in rock crevices where they were not observable. It is also possible that the smallest juveniles may have gone up into the water column during the night, although this was not evident from the s_A collected at night.

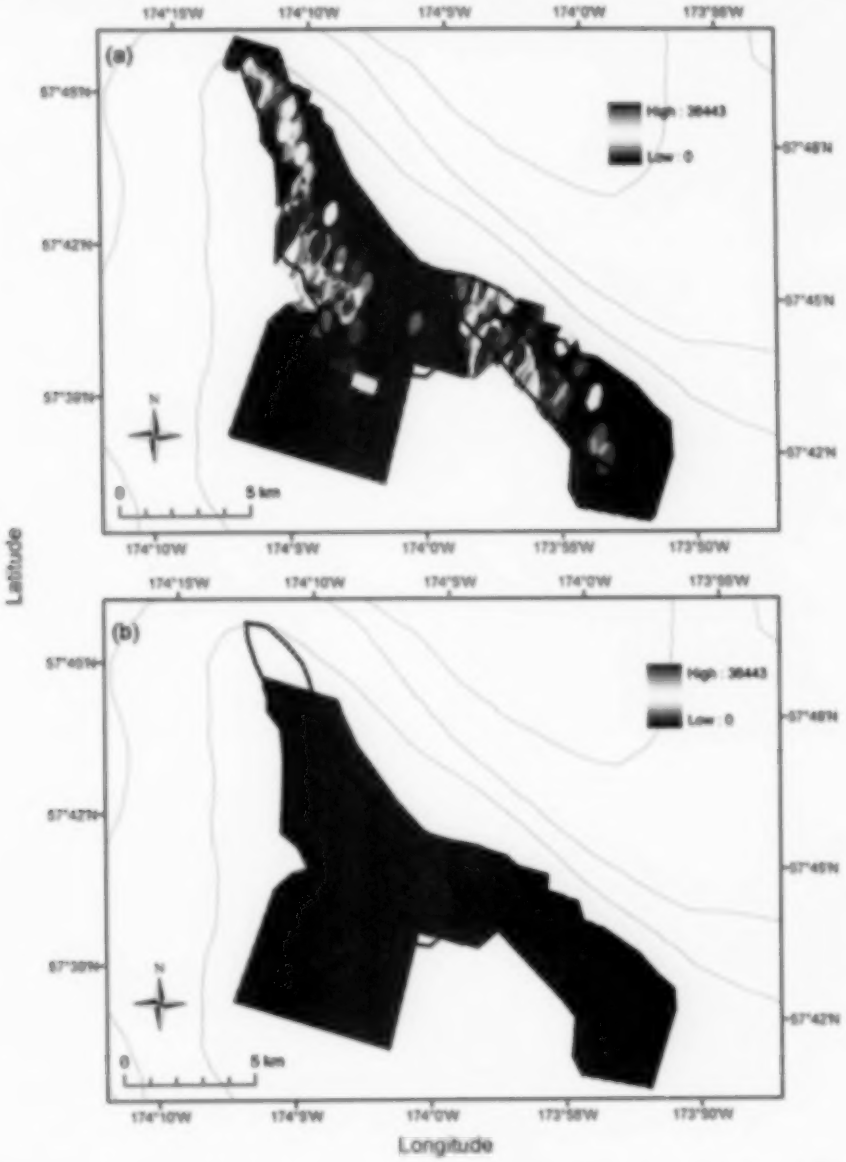
The distribution of rockfishes in the water column (indicated by s_A values) was uniformly low during nighttime surveys (as in Fig. 8). In contrast, daytime surveys indicated significant hotspots of fish density on the southern edges of both the north and south ridges (Fig. 6). In particular, the s_A values measuring fish in the water column were roughly two orders of magnitude larger during the day than during the night (Fig. 5). This corresponds to the magnitude of the change in adult rockfish density that was observed on the seafloor in the video transects, except in reverse.

The estimated numbers of juvenile and adult fish in the water column (from 2.5 m off the bottom to 40 m below the surface) from the acoustic surveys were 15.4 and 41.6 million, respectively, during the first daytime survey. In the second daytime survey, the number of juveniles observed in the water column was 16.3 million, whereas the number of adults was 44.0 million. Based on the paired day–night video transects, the additional number of juvenile rockfishes observed on the seafloor during the nighttime (in excess of what was observed during the day) was 9.6 million. The additional number of adult rockfishes on the seafloor during the nighttime video transects was 32.9 million. This provides strong evidence that the fish observed in the water column during the day moved to the seafloor at night.

Biomass estimate

The weighted mean length of a rockfish in the water column based on the differences in length observed between

Fig. 6. Kriged distribution of nautical area scattering coefficient (s_A, $m^2 \cdot nmi^{-2}$) in the water column (from 2.5 m off the bottom to 40 m below the surface) during (*a*) day and (*b*) night acoustic surveys of Zhemchug ridges. The dark outlines indicate the extent of the north and south ridges.

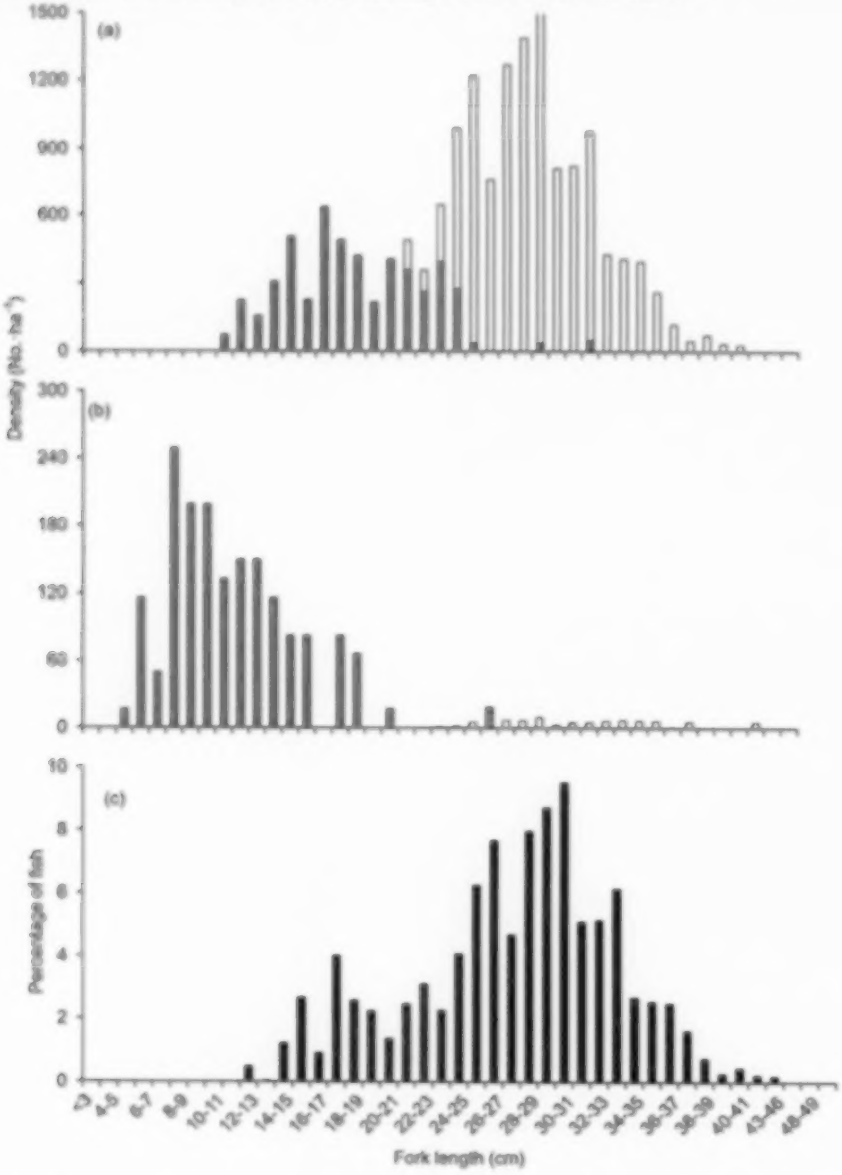


night and day in the video was estimated to be 27.1 cm. Using the length distribution and species composition data and the integrated s_A value from the two daytime surveys of each ridge, the calculated biomass of fish for the combined rocky ridge areas of Zhemchug was 1.59×10^4 t during the first daytime survey and 1.68×10^4 t during the second daytime survey. The two surveys varied by less than 6% in their estimates of total rockfish biomass, a characteristic reflected in the relatively narrow confidence intervals for the biomass estimates. The average (of the two daytime surveys) biomass of juvenile fish was estimated to be 9.2×10^2 t (95% confidence interval, ±49 t), and the average biomass of adult fish was estimated at 1.54×10^4 t (95% confidence interval, ±825 t). The vast majority of the biomass was comprised of adult rockfishes (96%), which were primarily northern rockfish (97%).

Discussion

Rockfishes inhabit a wide variety of seafloor habitats, with some species commonly found on soft substrate habitat on the continental slope in Alaska and the western coast of the US and Canada. In particular, species such as green-striped rockfish (*Sebastes elongates*), adult Pacific ocean

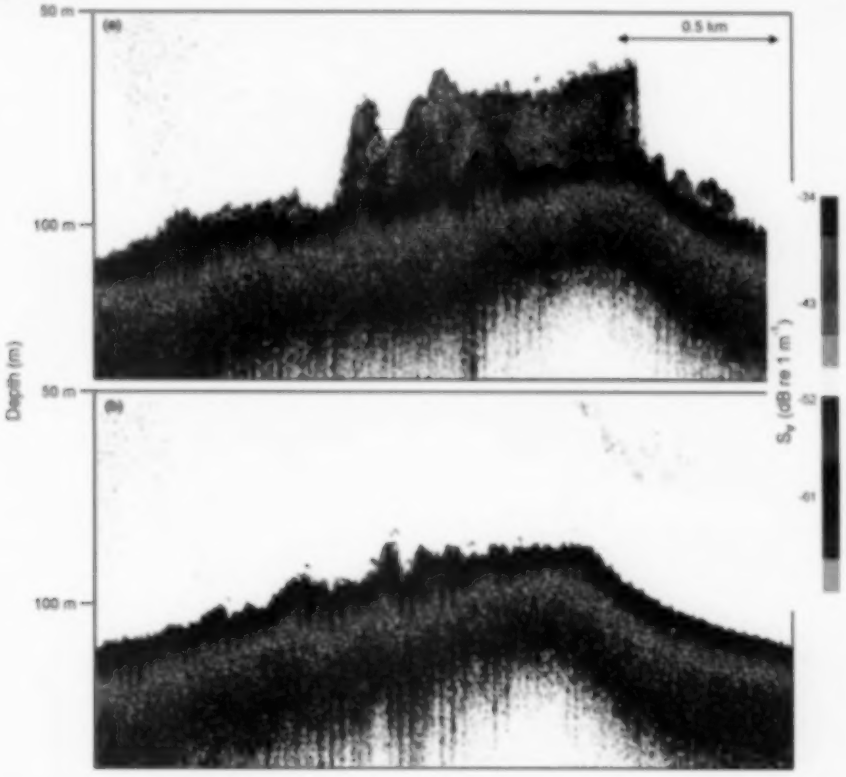
Fig. 7. Length–frequency plots of juvenile (shaded bars) and adult (open bars) (97% northern rockfish) rockfishes identified and measured from the video during (*a*) two nighttime underwater camera transects and (*b*) two daytime transects at the same location; (*c*) the percentage of fish assumed to be in the water column during the day (by fork length) is also shown (solid bars).



perch, rougheye rockfish (*Sebastes borealis*), and shortraker rockfish (*Sebastes aleutianus*) are often observed in soft substrates (Krieger and Ito 1999; Brodeur 2001; Tissot et al. 2007), and there is some evidence that shortspine thornyhead (*Sebastolobus alascanus*) prefer mud substrates over rocks (Stein et al. 1992; Jacobson and Vetter 1996; Lauth et al. 2004). Occurrence of these species on soft substrates makes them easier to survey using bottom trawl gear and, as such, may not cause as large a problem for fisheries management as species with affinities for rocky areas. For this study, it was important to show that the species that we observed (juvenile Pacific ocean perch and northern rockfish) were found exclusively in high-relief habitat so that we could make an estimate of their biomass, knowing the areal extent of the rocky ridges.

The affinity of juvenile Pacific ocean perch and other juvenile rockfish species for high relief seafloor demonstrated in this study is similar to that reported in other areas (Carlson and Haight 1976; Pearcy et al. 1989; Rooper et al. 2007). The observed affinity of adult northern rockfish is also not unexpected given that evidence in the literature suggests that rocky outcroppings such as the Zhemchug ridges

Fig. 8. Acoustic volume backscatter strength (S_v, dB re 1 m^{-1})) from transects over the Zhemchug ridges showing (*a*) daytime and (*b*) nighttime volume backscatter in the water column from 40 m below surface to the seafloor. Data were collected using a calibrated 38 kHz Simrad ES-60 echosounder.



should be important habitat for northern rockfish. In fact, the spatial distribution of catches in the commercial fisheries in the Gulf of Alaska and Aleutian Islands shows that northern rockfish prefer isolated shallow banks near the shelf break, with the southeast Zhemchug area historically accounting for 62% of all northern rockfish taken by commercial fishery bottom trawls in this region (Clausen and Heifetz 2002).

However, the size of the rockfish population at the Zhemchug ridges was surprising considering the paucity of rockfishes in other areas of the eastern Bering Sea shelf. The estimated biomass from the relatively small (56.9 km^2) Zhemchug ridges area dwarfs the total estimated biomass of northern rockfish from both eastern Bering Sea slope bottom trawl surveys in 2004 (1.59 t) and 2008 (3.15 t) (Hoff and Britt 2005; Hoff and Britt 2009), as well as the total rockfish biomass from the eastern Bering sea shelf bottom trawl survey in 2007 (8079 t; Acuna and Lauth 2008). The northern rockfish observed on the Zhemchug ridges occurred in an area not surveyed by either of those bottom trawl surveys as they cannot be prosecuted in high-relief rocky ridge habitat. The two bottom trawl surveys may thus significantly underestimate the biomass of northern rockfish in the eastern Bering Sea if similar rocky ridges occur at other locations along the outer continental shelf.

The biomass of rockfishes estimated from this study (1.64×10^4 t) was higher than that of Stanley et al. (2000), who estimated a total biomass of widow rockfish of 1000–2630 t in a 21–28 km^2 area (roughly one-half the area of the Zhemchug ridges) off British Columbia. About 1100 t of yellowtail rockfish (*Sebastes flavidus*) were also estimated using acoustics in a 100 km^2 area (Stanley et al. 1999) in British Columbia. The coefficients of variability estimated from the two daytime acoustic surveys in our study (4%) for juvenile and adult rockfishes were substantially less than those estimated from other rockfish surveys (14%–30%; Gunderson 1997; Stanley et al. 1999; Ralston et al. 2003). This is probably due to the small sample size ($n = 2$ surveys) used to estimate the variance. The relatively short time over which our data was collected probably also reduced variance associated with taking multiple surveys of the population.

The size of northern rockfish was similar to that found in Aleutian Islands surveys, with fish length in this study ranging from 9 to 41 cm (mean length = 30 cm). In three Aleutian Islands surveys examined by Clausen and Heifetz (2002), the mean size of northern rockfish was 29.9 cm and ranged from 15 to 38 cm. The size frequency of juvenile Pacific ocean perch observed from video observations on the Zhemchug ridges suggests that these features support a different portion of the population than is present in the rest of the eastern Bering Sea shelf and slope. Length data from the eastern Bering Sea slope and shelf bottom trawl surveys show that most individuals captured in the bottom trawl survey are adults (>250 mm fork length, FL) (Hoff and Britt

2005; Acuna and Lauth 2008). In contrast, this study showed that the Zhemchug ridges are occupied primarily by immature Pacific ocean perch, as mature adults were not detected in any large numbers. This distinct habitat association of young Pacific ocean perch suggests that the Zhemchug ridges may be the first juvenile Pacific ocean perch habitat documented for the eastern Bering Sea and an important nursery location necessary for successful recruitment, as has been suggested for similar habitats in the Gulf of Alaska and Aleutian Islands (Rooper and Boldt 2005; Rooper et al. 2007).

The diel patterns in rockfish behaviour were similar to those found by Brodeur (2001), with rockfishes moving into the water column during the day and moving to the seafloor at night. Brodeur (2001) observed Pacific ocean perch in the water column of Pribilof Canyon during daylight hours when they were presumably feeding on euphausiids and then retreating to the cover of sea whip forests during the nighttime. However, this is a different behavior than has been previously observed in semipelagic schooling rockfishes. On the US West Coast, daytime pelagic behaviour is not as common, whereas nighttime forays into the water column are more prevalent (Stanley et al. 2000).

There were a number of sources of potential error in our estimation of the total biomass of rockfishes (and its variance) at the Zhemchug ridges. Notably, species identification was difficult using the video footage. Although adult rockfishes were easily distinguished by species, the juvenile rockfishes were not always easily identified to species. Potential errors in size estimation would affect our biomass estimates. Although the camera calibrations before and after the project were accurate and the fish sizes observed were similar to size frequencies from trawl surveys in surrounding areas, the absence of physical collection of specimens meant that true verification of size estimates was not possible. We also chose not to extend the acoustically estimated biomass into the acoustic "dead zone" where the camera observed few adult or large juvenile fish in the daytime. This probably resulted in a conservative estimate of total biomass. By far the largest source of potential error in the biomass estimate was the literature value used for target strength. We chose this relation based on the review of Stanley et al. (2000), and this relationship was used for studies of widow rockfish off British Columbia. This target strength relation is close (0.2 dB higher) to that estimated more recently for captive *Sebastes schlegeli* (Kang and Hwang 2003). Other recent estimates of target strength for Atlantic redfish (*Sebastes* spp.) have been 0.6 dB (Gauthier 2001) and 1.2 dB (Gauthier and Rose 2002) higher than that of Stanley et al. (2000). Using the Gauthier and Rose (2002) relationship would result in 32% more rockfishes than obtained in our results. The sources of error could be reduced in future studies by estimating an accurate target strength for northern rockfish and Pacific ocean perch and using a method of capture that would allow verification of size frequencies that are observed in the video.

This research produced a habitat-based biomass estimate for fishes in an untrawlable area using acoustics as the primary tool for measuring fish biomass and stereo video for estimating both species composition and fish length. We estimated 1.54×10^4 t of adult rockfishes (predominantly northern rockfish) and 9.2×10^2 t of juvenile rockfishes (predominantly Pacific ocean perch). Adult fish and larger juveniles were in the water column during the daylight hours and on bottom during nighttime. Smaller juveniles were found on the seafloor during daytime hours. While on bottom, all rockfishes were found in rocky ridge habitats and rarely on sandy flat seafloor. Rockfishes in the water column were found predominantly over the rocky ridges rather than over the flats. Utilization of similar survey methodologies on a larger scale may improve assessment of rockfishes not only in Alaska, but also throughout their range where fishery-independent biomass estimates have been difficult to obtain.

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The influence of the Columbia River plume on the survival of steelhead (*Oncorhynchus mykiss*) and Chinook salmon (*Oncorhynchus tshawytscha*): a numerical exploration

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Abstract: Are smolt-to-adult return rates (SARs) for wild steelhead (i.e., sea-run rainbow trout, *Oncorhynchus mykiss*) and wild Snake River spring–summer Chinook salmon (*Oncorhynchus tshawytscha*) related to changes in the Columbia River plume at the time that juvenile migrants enter the ocean? We used three-dimensional (3D) numerical models of the baroclinic circulation in the Columbia River estuary–plume–shelf system to simulate within-season variation in plume size and location, comparing the results with SARs for each day that juvenile salmon entered the ocean for 1999–2003. We found that steelhead benefited from the plume environment at a narrow window of time around their ocean entry. However, when large-scale ocean conditions turned unfavorable, the contribution of local plume conditions to the overall variability in steelhead survival became not significant. A similar evaluation revealed that the plume did not affect survival of Chinook salmon, at least at the fine scale of variability considered. The differential response between the two species is consistent with observed and previously reported behavioral characteristics they exhibit. We speculate that steelhead mainly use the plume to move quickly away from coastal habitats and the predation pressures associated with this environment, for a more direct migration than Chinook salmon to ocean habitats in the Gulf of Alaska.

Résumé : Les taux de survie du saumoneau à l'adulte (SARs) des truites arc-en-ciel anadromes (*Oncorhynchus mykiss*) sauvages et des saumons chinook (*Oncorhynchus tshawytscha*) de printemps–été de la Snake sont-ils reliés aux changements dans le panache du Columbia au moment où les jeunes saumons pénètrent dans l'océan? Nous avons utilisé des modèles numériques tridimensionnels (3D) de la circulation baroclinique dans le système estuaire–panache–plate-forme du Columbia pour simuler la variation au cours de la saison de la taille et de la position du panache, en associant les résultats aux SAR pour chaque jour où les jeunes saumons ont pénétré dans la mer en 1999–2003. Les truites arc-en-ciel anadromes ont tiré bénéfice de l'environnement du panache pendant une courte période au moment de leur arrivée dans l'océan. Cependant, lorsque les conditions océaniques à grande échelle sont devenues défavorables, la contribution des conditions du panache local à la variabilité globale de la survie des truites arc-en-ciel anadromes est devenue non significative. Une évaluation similaire a montré que le panache n'affecte pas la survie des saumons chinook, au moins à l'échelle fine de la variabilité considérée. Les réponses différentes des deux espèces sont en accord avec les caractéristiques comportementales observées présentement et signalées antérieurement chez elles. Nous croyons que les truites arc-en-ciel anadromes utilisent le panache pour s'éloigner rapidement des habitats côtiers et des pressions de prédation qui leur sont associées, afin de migrer plus directement que les saumons chinook vers les habitats océaniques dans le golfe de l'Alaska.

[Traduit par la Rédaction]

Introduction

The Endangered Species Act listing of many evolutionarily significant units of eastern Pacific Ocean salmon (*Oncorhynchus* spp.) has prompted extensive research into possible causes for their declines. Although most research on salmon mortality historically focused on fresh water, both freshwater and ocean environments contribute substantially to egg-to-adult mortality, and they contribute roughly equally to the interannual variability in such mortality (Bradford 1995). Additionally, a considerable body of evidence exists that indicates that for the ocean phase of sal-

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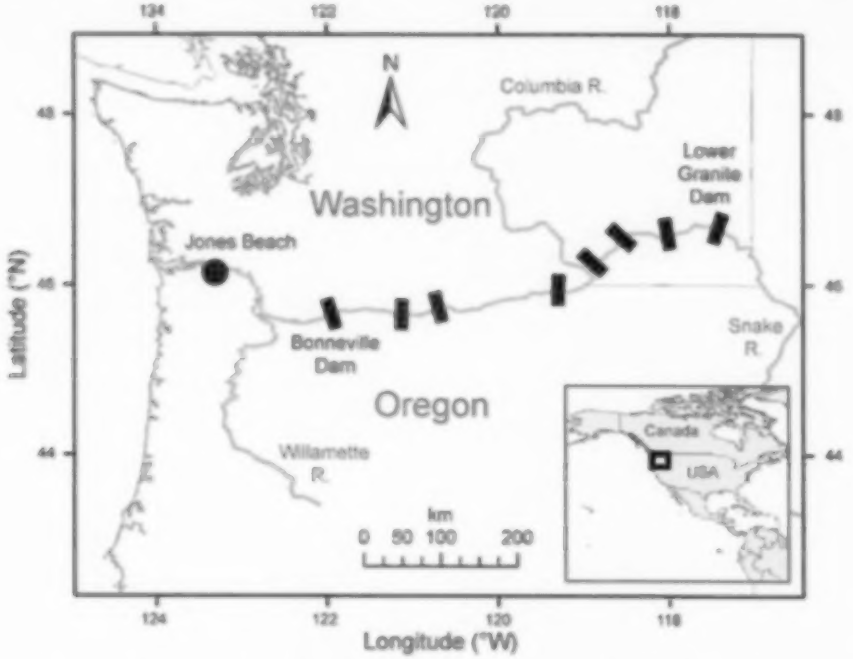
Fig. 1. The Columbia River basin in Oregon and Washington, USA. Shown are the locations of the tagging site, Lower Granite Dam, where smolts are loaded on barges for transportation through the dam system, and of Bonneville Dam, downstream of which fish are released back into the river.



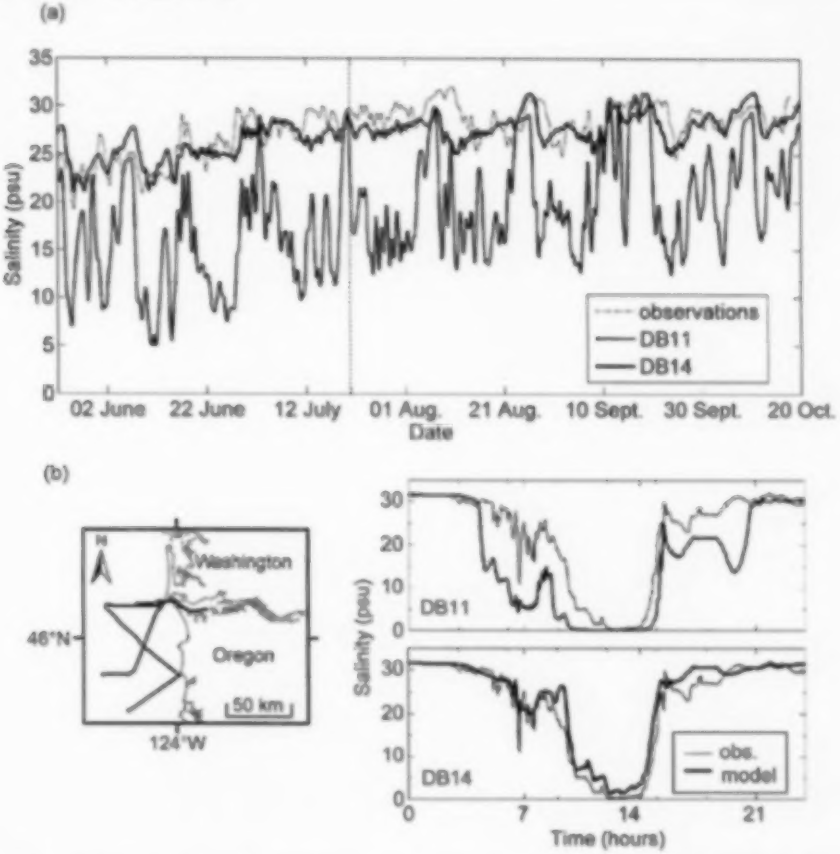
mon life history, most mortality occurs within the first few weeks or months of ocean residence (Pearcy 1992).

In recent years, more effort has gone into understanding the relationship between Pacific salmon production and climate variability patterns, such as the El Niño Southern Oscillation (ENSO) and the Pacific Decadal Oscillation (PDO) (Johnson 1988; Mantua et al. 1997; Peterson and Schwing 2003). Local marine environments, as affected by ocean shelf upwelling or infusion of fresh water from river plumes may play as large a role in the early marine survival of salmon as the regime shifts operating at broader regional scales (Beamish et al. 1994; Peterman et al. 1998; Casillas 1999).

Recent field research has begun to develop information that provides an understanding of the mechanisms by which the plume of the Columbia River may influence juvenile salmon survival (Emmett et al. 2004; De Robertis et al. 2005; Morgan et al. 2005). Emmett et al. (2004) found that the highest densities of subyearling Chinook salmon (*Oncorhynchus tshawytscha*) off the Columbia River were associated with the stronger currents emanating from the river mouth during ebb tide, while densities of yearling Chinook salmon were higher at lower water clarity. They also found that in the study location, most juvenile salmon resided in the upper 12 m of the water column. They recognized the possibility that features associated with the plume (low salinities, high turbidity, fronts, and eddies) determined this distribution. De Robertis et al. (2005) confirmed these suggestions, as they found a higher abundance of juvenile Pacific salmon in the coastal region off the Columbia River associated with the low-salinity plume waters and frontal zones compared with the surrounding, more saline ocean waters. They called for focused studies to determine if growth and survival of juvenile salmon are related to plume structure and, in particular, to changes in the timing and intensity of freshwater discharge from the Columbia River into the coastal ocean. Morgan et al. (2005) focused on the hypothesis of plume fronts as feeding habitats for juvenile salmon. Fronts were found to be zones of increased zooplankton biomasses. However, based on stomach content analysis, juvenile salmon do not seem to take advantage of this valuable food resource, possibly because of the transience of the frontal features or the small scale of the frontal regions compared with the size of the plume and ocean habitats. Research is starting to address the intra-annual variability in survival rates of steelhead (i.e., sea-run rainbow trout, *Oncorhynchus mykiss*) and yearling Chinook salmon leaving the Columbia River system (Scheuerell et al. 2009). The rapidity of change in survival suggested local conditions in the environments that connect the river migration corridor to the ocean were more likely a determinant of the observed variability than conditions farther away (in the ocean feeding areas of the Gulf of Alaska or Bering Sea).

Initial results from this recent research suggest that the survival of outmigrating juvenile salmon varies at time scales consistent with changes in the Columbia River plume. Such changes occur not just across seasons and years, but also on a time scale of days (Hickey et al. 1998, 2005). With knowledge that the plume varies temporally within a migration season as well as inter-annually (Hickey et al. 1998, 2005), and with multiyear databases of baroclinic circulation in the Columbia River estuary–plume–shelf system from the CORIE (Columbia River Ecosystem) coastal-

Fig. 2. The quality of CORIE simulations is routinely assessed through comparisons with real-time observations from the network of sensors deployed in the Columbia River estuary and on buoys off the river mouth, as well as with data collected by cruises of opportunity. Shown are model-data comparisons, for salinity simulations from the two circulation databases, DB11 and DB14, generated with the models ELCIRC and SELFE, respectively (the latter being the default model in the study, while the former was used to explore sensitivity to model algorithmic solutions and parameterization): (*a*) at the OGI02 buoy (reference position 46°10.407′N, 124°7.630′W), at 1 m depth, offshore the river mouth (time series are low-pass filtered); and (*b*) along a cruise path (21 July 2004). The dotted vertical line in (*a*) identifies the day of the same cruise in the OGI02 time series.



margin observatory (Baptista 2006), we undertook a correlation analysis to relate the variability in the physical plume environment to the variability in salmon survival.

Materials and methods

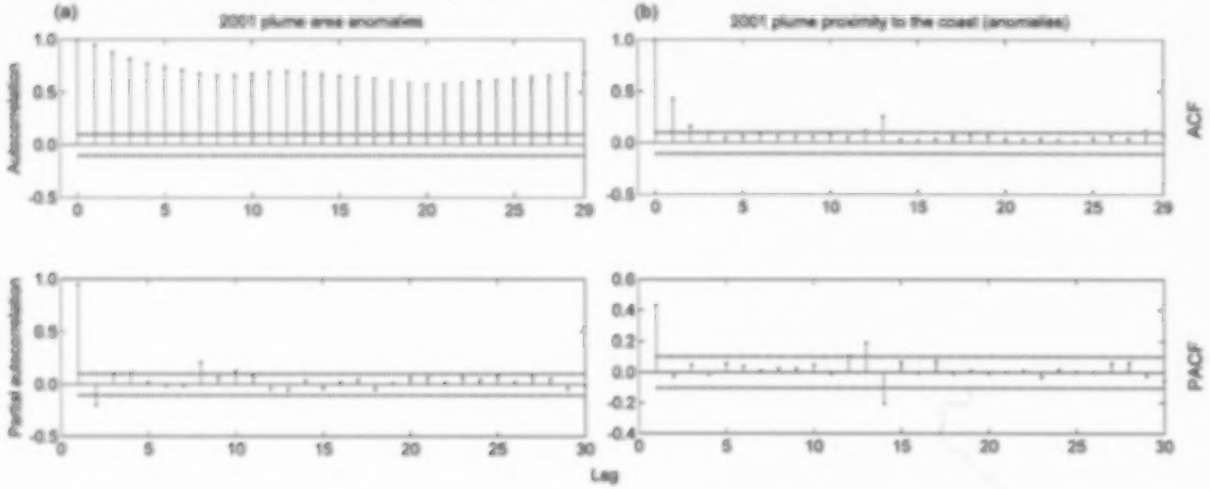
Study area

The Columbia River (Fig. 1) is the largest river on the US Pacific coast, with daily discharges ranging, over a typical year, from 3200 to 10 500 $m^3 \cdot s^{-1}$, with recent extremes of 1800 $m^3 \cdot s^{-1}$ in 2001 and of 24 500 $m^3 \cdot s^{-1}$ during 1996 (US Geological Survey National Water Information System: http://waterdata.usgs.gov/or/nwis, accessed 25 January 2009). The Columbia River plume is a dominant hydrographic feature off the Oregon and Washington coasts.

Model simulations of the Columbia River plume physical environment

To characterize variability in the structure of the Columbia River plume at the time juvenile salmon migrants enter the ocean, we used multiyear (1999–2006) simulations of the 3D salinity field from the CORIE coastal-margin observatory (Baptista 2006). Developed in 1996 for the Columbia River estuary–plume–shelf system, CORIE integrates an observation network with the simulation capabilities of a modeling system and with a web-based information system. The modeling component of CORIE relies on two alternative numerical models that can be used to simulate 3D baroclinic circulation in the Columbia River estuary–plume–shelf system: an older model (ELCIRC; Zhang et al. 2004; Baptista et al. 2005) and a more recent one (SELFE; Zhang and Baptista 2008). Key differences between the two are the numerical algorithm used to solve the set of equations that describe circulation in the system and a different solution for the vertical discretization of the computational domain. ELCIRC is a finite difference – finite volume model, while SELFE uses a finite element framework, with terrain-following coordinates near the bottom, versus the unstretched z coordinates used by ELCIRC. Because ELCIRC tends to predict salinities that are systematically lower than observations in the

Fig. 3. Autocorrelation (ACF) and partial autocorrelation (PACF) functions for the anomalies in (*a*) plume surface area in 2001 and (*b*) plume proximity to the coast in 2001 ($n = 365$; $\alpha = 0.05$). The 95% confidence interval is shown. The *x* axis indicates the number of lags. The shape of the ACF in (*a*) reveals the nonstationarity exhibited by the plume surface area (the same was observed for the plume volume, both in 2001 and in 1999 — not shown). Firstdifferencing did not eliminate the autocorrelation, suggesting more complex autoregressive integrated moving average (ARIMA) models. Even where there was no indication of nonstationarity for the same metrics in other years, differencing once was not enough to eliminate autocorrelation because of the higher order autoregressive properties of the time series. Isolated spikes at higher lags in the PACF of the plume proximity to the coast (*b*), in particular in a year of low river discharge such as 2001, revealed the periodicity linked to the fortnight tidal signal (spring–neap cycle).



Columbia River plume region (Fig. 2), we used SELFE as the default model for the highly resolved, long-term simulations, and the bulk of the results presented in this paper used a circulation database (DB14) generated with SELFE. However, SELFE has not yet proven to outperform ELCIRC in all regards. So to explore the sensitivity of our results to model uncertainty arising from specific algorithmic solutions and model parameterizations, we repeated the correlation analysis (described below) using a realization of the circulation database (DB11) generated with ELCIRC.

Both models predict water levels, 3D velocities, salinity, and temperature at each point in space and time of the computational domain. For the purpose of this study, we translated this output in terms of metrics of plume size (area of the surface plume and volume) and location (expressed in terms of coordinates of the centroid of the surface plume). We defined the plume using a cutoff salinity of 28 psu, because it provides a conservative choice to distinguish the body of Columbia River plume waters from other freshwater sources and from ambient ocean salinity.

Salmon data

As an indicator of varying population performance in changing estuary, plume, and ocean-shelf conditions, we used smolt-to-adult return (SAR) rates computed from passive integrated transponder (PIT) tag data. We used PIT-tag data from Snake River spring–summer Chinook salmon (*O. tshawytscha*) and steelhead (*O. mykiss*), originally collected for studies designed for purposes other than this analysis. The SARs were computed by dividing the number of adult PIT-tagged fish returning to Lower Granite Dam (river kilometre (rkm) 695) by the number of juveniles previously collected, tagged at, and transported from there on barges to a release site downstream of Bonneville Dam (rkm 234) (Fig. 1). The SARs are therefore a rate of survival over a period of time that encompasses the 2–4 years of ocean residence as well as the upriver migration when adult salmon return to spawn.

We used SARs from the 1999, 2001, 2002, and 2003 outmigrations of (yearling) wild Snake River spring–summer Chinook salmon and wild steelhead (see Appendix A), both evolutionarily significant units listed as threatened under the Endangered Species Act. No data exist for the same transport group (fish collected, tagged, and barged at Lower Granite) for 2000. The data in each year covered the salmon outmigration from the beginning of April through mid-June.

The data are referenced to the day the smolts are loaded on barges after tagging. Day of ocean entry of the juvenile migrants was estimated by adding 36–48 h for barging the fish past the dam system, about 2 days for the smolts to migrate to Jones Beach (rkm 75) and then 2 more days to leave the estuary and enter the plume.

Preprocessing of the time series of SARs separately computed for the smolts tagged on each day was limited to filtering once with a three-point median filter, to reduce gaps in the SAR series (which typically preceded or followed dates on which fish were barged early or late in the day) and to smooth the most extreme spikes.

Correlation analysis

We conducted a correlation analysis between SARs separately computed for the smolts tagged on each day and daily plume metrics because the Columbia River plume is a fast-changing hydrodynamic feature. It responds nearly instanta-

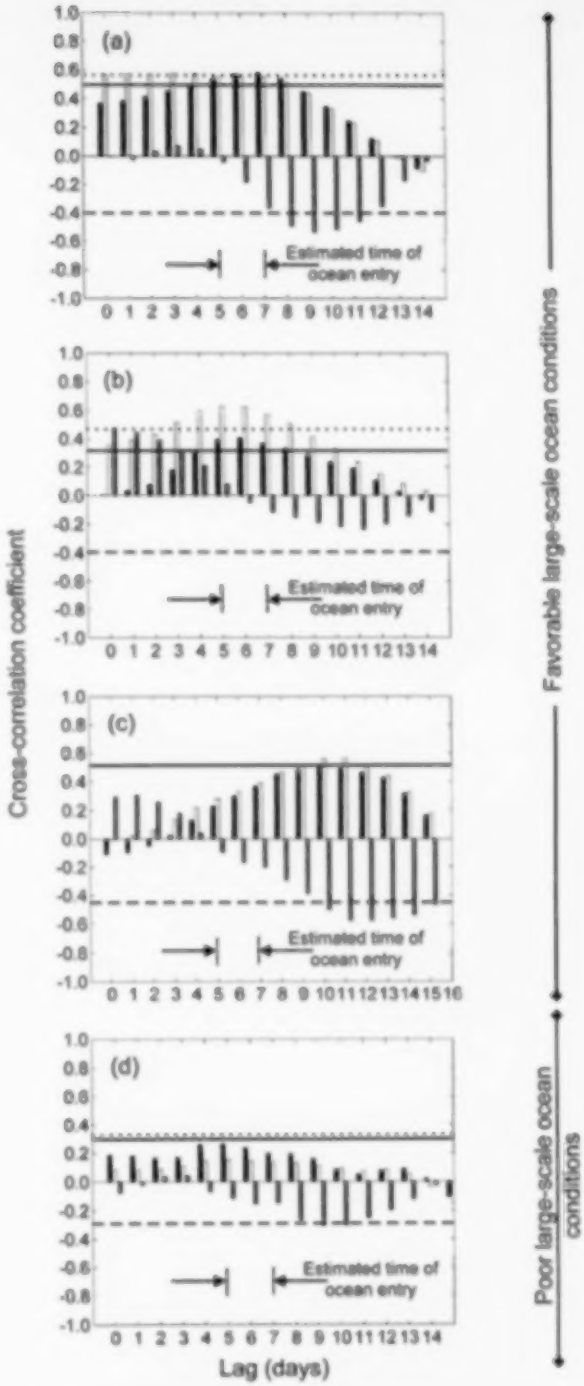


Fig. 4. Cross-correlations between anomalies in steelhead SARs and plume metrics, as a function of the lag by which the plume time series are imposed to trail the SARs in the correlation: (*a*) 1999; (*b*) 2001; (*c*) 2002; (*d*) 2003. Black bar: plume area; white bar: plume volume; gray bar: proximity to the coast. Lag zero corresponds to the day of fish tagging and collection upriver. The figure shows the approximate lag at which SARs are being correlated with the plume conditions existing at the estimated time of ocean entry of juveniles. At shorter lags the fish are still in river, either being transported through the dam system or migrating through the estuary. Horizontal lines show critical values for statistical significance at the 5% nominal level (significance of peak correlations is tested; solid line: plume area; dotted line: plume volume; dashed line: proximity to the coast). Total number of lags considered was chosen based upon the length of the available SAR data set.

neously (within hours) to shifts in wind regimes — with wind fluctuations having time scales of 2–10 days — while the plume response to river discharge is modulated by the semidiurnal and diurnal tides (Hickey et al. 1998). Maps of the daily averaged simulated salinity field that capture the variability determined by the fast dynamics of the plume on the Washington and Oregon shelf can be found in Burla et al. (2010).

Although models could produce highly resolved temporal scales for plume characteristics, we could only roughly estimate the time juvenile salmon left the estuary and entered the ocean as 5–6 days after the day on which fish were tagged and barged for transportation. To identify when the cross-correlation between SARs and plume metrics reaches its peak with respect to the time fish were tagged upriver and to the time of estimated ocean entry, we explored the cross-correlations at different time lags (lagging the plume time series by an increasing number of days relative to the SAR series).

To limit the linearity assumption that is implicit in a correlation analysis, we performed the analysis using anomalies — limiting the assumption to the relationship between the stochastic deviations in the variables (here from a baseline provided by a multiyear climatology), rather than between the variables themselves (Thiébaux 1994). Daily anomalies in the plume size and location, relative to climatologies (multiyear weekly averages) computed from the long-term computer simulations, were meant to capture how the variability in the plume structure relates to a similarly defined variability in salmon survival. Salmon survival data were available only for 1999, 2001, 2002, and 2003. Hence we constructed the climatologies based upon these 4 years only, not only for the SARs but also for the plume metrics.

Testing the statistical significance of the correlations accounting for the autocorrelation in the data

To test the significance of the cross-correlations between salmon survival rates and plume metrics and account for the autocorrelation in the data, we adopted a nonparametric method from the climate and physical research literature (Prichard and Theiler 1994; Ebisuzaki 1997). The advantage of the nonparametric test lies in its simplicity, in avoiding the task of fitting a statistical model, and more importantly, in avoiding the assumptions other approaches rely upon or implicitly make (Pyper and Peterman 1998). The shape of the autocorrelation (ACF) and partial autocorrelation (PACF) functions (Fig. 3) suggested that simple autoregressive models of the first order, often a good approximation in fisheries series, were not adequate to describe the plume

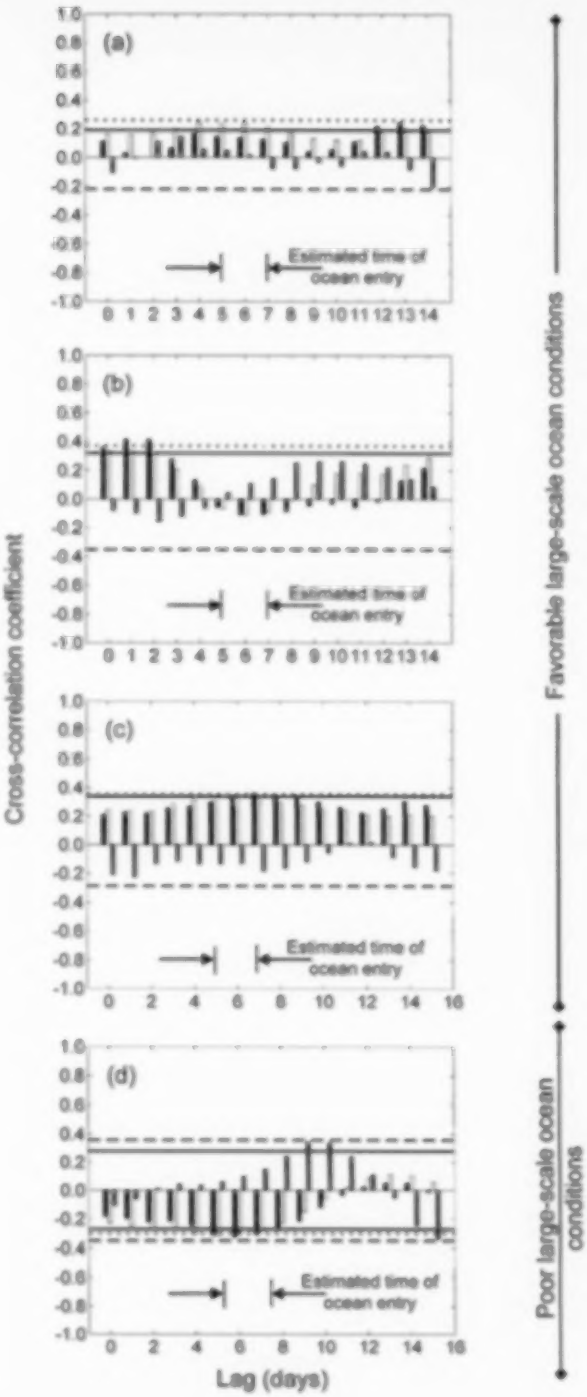


Fig. 5. Cross-correlations between anomalies in Chinook salmon SARs and plume metrics, as a function of the lag by which the plume time series are imposed to trail the SARs in the correlation: (*a*) 1999; (*b*) 2001; (*c*) 2002; (*d*) 2003. Black bar: plume area; white bar: plume volume; gray bar: proximity to the coast. Lag zero corresponds to the day of fish tagging and collection upriver. The figure shows the approximate lag at which SARs are being correlated with the plume conditions existing at the estimated time of ocean entry of juveniles. At shorter lags, the fish are still in river, either being transported through the dam system or migrating through the estuary. Horizontal lines show critical values for statistical significance at the 5% nominal level (significance of peak correlations is tested; solid line: plume area; dotted line: plume volume; dashed line: proximity to the coast). Total number of lags considered was chosen based upon the length of the available SAR data set.

metrics time series in our study. Hence, we could not remove autocorrelation by first-differencing. In addition, while we used a daily sampling frequency to be able to capture the fast-changing dynamics of the Columbia River plume, we could not assume that lower frequencies of variability, which are removed by removing the autocorrelation, were unimportant. Because of the size of our SAR data set and the nonstationarity exhibited by some of the plume metrics series (Fig. 3), we also had concerns about the potential shortcomings of adjusting the procedure to test the null hypothesis of no correlation by replacing the sample size with the "effective" number of degrees of freedom (Pyper and Peterman 1998; Botsford and Paulsen 2000).

The nonparametric method we adopted consisted of determining the critical value for the significance of the cross-correlations between SARs and plume metrics, r_{crit}, by generating an empirical sampling distribution for the cross-correlation coefficients, "resampling" in the frequency domain. "Resampling" in the frequency domain exactly preserves the power spectrum, and hence resampled series (surrogates) retain the autocorrelation structure of the original series (Ebisuzaki 1997), which traditional bootstrapping techniques may fail to replicate (Zwiers 1990).

To generate the surrogate time series that we used to estimate the empirical distribution for the cross-correlations in our analysis, we followed Prichard and Theiler (1994). We first obtained the discrete Fourier transform of the original time series $x(t)$ from

$$(1) \qquad X(f) = F[x(t)] = \sum_{n=0}^{N-1} x(t_n)e^{2\pi i f t_n \Delta t}$$

for $t = t_0, t_1, \ldots, t_{N-1} = 0, \Delta t, \ldots, (N-1)\Delta t$. This can be rewritten as

$$(2) \qquad X(f) = |X(f)|e^{i\phi(f)}$$

which is evaluated at the discrete frequencies $f = -N\Delta f/2, \ldots, -\Delta f, 0, \Delta f, \ldots, N\Delta f/2$, where $\Delta f = 1/(N\Delta t)$. We generated a "phase-randomized" Fourier transform by rotating the phase ϕ at each frequency by an independent random variable φ, drawn from a uniform distribution in the range $(0,2\pi)$:

$$(3) \qquad \tilde{X}(f) = |X(f)|e^{i[\phi(f)+\varphi(f)]}$$

To ensure that the inverse Fourier transform was a time series of real values, φ was constrained to satisfy the symmetry property $\varphi(f) = -\varphi(-f)$.

The inverse Fourier transform gave the surrogate time series

(4) $\tilde{x}(t) = F^{-1}\{\tilde{X}(f)\}$

We repeated steps (3–4) to generate 2000 surrogates of the time series for the plume metrics and to compute the critical value for the significance of the cross-correlations with the SARs, r_{crit}, as the 95th percentile (5th percentile for negative correlations) of the generated distribution. We applied the resampling to the time series of the plume metrics, which, contrary to the observed SARs, had no gaps, being computed from the simulations of the circulation model. Taking the 95th percentile corresponded to testing at the 0.05 significant level in a one-tail test.

Results

Data from migration years 1999, 2001, and 2002 indicated a consistent pattern in the correlations between the anomalies predicted by the plume modeling and anomalies in the survival rates for steelhead. Data from 2003 did not. When plotted as a function of the lag between the two time series (with the plume metrics series trailing), the cross-correlations between anomalies in steelhead SARs and daily-averaged plume metrics (Fig. 4) consistently peaked shortly after the estimated time of ocean entry of the outmigrating juveniles. The cross-correlations with plume size were consistently positive, i.e., positive anomalies in plume volume and area (a larger plume, in particular a larger surface plume) correlated with increases in steelhead survival throughout each migration season. The cross-correlations with the plume location relative to the coast were consistently negative. The plume proximity to the coast was represented by the location in the east–west direction of the plume centroid. During the salmon outmigration season, the climatology showed a plume prevalently detached from the coast and oriented to the southwest (but with variability around this known seasonal pattern). Small or negative anomalies in the centroid metric correlated with increases in steelhead survival. Anomalies of this sign correspond to a plume that is either located where predicted by the climatology or extending farther offshore.

The strength of the correlations, at the peak values, was moderate in the first 3 years of the study, leading to a proportion of variability in the steelhead SARs explained by the variability in plume structure that ranged from 16% to 40%, except for the plume proximity to the coast in 2001, in which case it amounted to only 5%. The strongest correlation was in 2001 with plume volume anomalies (0.63). Although in most cases correlations passed the nonparametric test for statistical significance, they did so convincingly only in 2001 with plume size and in both 1999 and 2002 with the plume proximity to the coast. However, the consistency in the correlation patterns was lost when we plotted the correlograms replacing the original plume time series with the surrogate time series, which we generated to construct the empirical distribution for the cross-correlation coefficients. This result indicates that the revealed patterns were not spurious and not merely due to the autocorrelation structure in the data, which the surrogate time series preserved.

In 2003, correlations still peaked around or after the time the juvenile steelhead were estimated to enter the ocean, with the same sign as in previous years. Correlations, however, were weak and not (or barely) statistically significant (weakly significant for the proximity to the coast).

Across all 4 years covered by the study, the cross-correlograms for Chinook SARs (Fig. 5) did not show the same distinct patterns, with weak, statistically nonsignificant correlations, suggesting that the influence of the plume environment is not significant for the survival of Chinook salmon, at least at the scale of variability considered in the analysis.

A comparison of the above results based on database DB14 (SELFE model) to results from database DB11 (ELCIRC model) provided relatively consistent predictions in the occurrence of the anomalies in the plume metrics. They often differed, however, in the order of magnitude and duration of the predicted anomalies and in the relative magnitude of the anomalies across one season. Consistently, the patterns observed in the correlations between steelhead SARs and plume anomalies from DB14 (Fig. 4) were largely confirmed with DB11 (not shown) but with correlations generally weaker than those obtained with DB14. We attribute both the consistency in the patterns and the different strength in the correlations with the SARs to the fact that ELCIRC systematically predicts overly fresh conditions in the plume region, a shortcoming that the algorithmic solutions in DB14 overcome (Fig. 2). Nevertheless, ELCIRC captures other important features of the plume behavior, in particular the plume response to shifts in wind regimes between upwelling- and downwelling-favorable conditions.

Discussion

Plume conditions and salmon survival

In addressing the question of whether the Columbia River plume, which is a dominant feature off the Oregon and Washington coasts, constitutes local ocean conditions that affect Pacific salmon production, we found that the fraction of the total variability that is intraseasonal in nature in the steelhead SARs relates to changes in the plume at the time the juvenile migrants enter the ocean. This relationship is not evident for Snake River spring–summer Chinook salmon. While observational studies have begun to address the same question (Emmett et al. 2004; De Robertis et al. 2005; Morgan et al. 2005), here we used an approach that relies on the simulation capabilities of the CORIE coastal-margin observatory and on biological observations taken in river for the purpose of other studies.

Although available observation and model data sets limited the study period to 4 years, they crossed a regime shift for the large-scale ocean conditions that allowed placing the effect of local plume conditions into context. Years 1999, 2001, and most of 2002 all fell within a short cool phase of the PDO that started at the end of the 1990s and ended in mid–late 2002 (Joint Institute for the Study of the Atmosphere and Ocean, Climate Impacts Group, http://jisao.washington.edu/pdo/PDO.latest, accessed 22 July 2008). They were therefore years characterized by cooler ocean conditions and enhanced biological productivity off the US west coast (Mantua et al. 1997; Hare et al. 1999; Peterson and Schwing 2003). Thus, the outmigrating juveniles during the first 3 years of this study all entered the ocean under favorable large-scale ocean conditions, which, however, soon

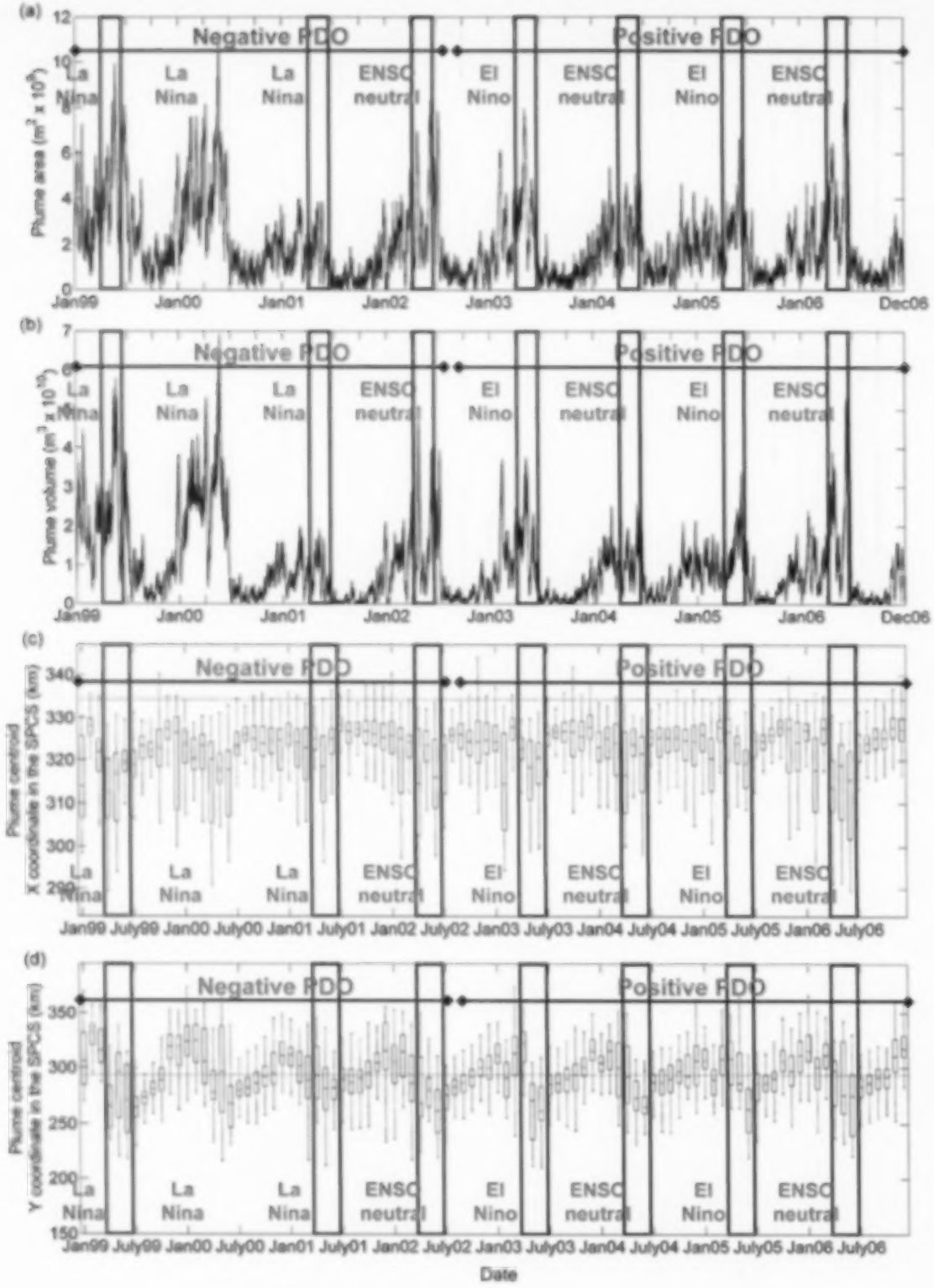

(a)
Negative PDO
Positive PDO
La Nina
La Nina
La Nina
ENSO neutral
El Nino
ENSO neutral
El Nino
ENSO neutral
Plume area (m^2 x 10^9)
Jan99 Jan00 Jan01 Jan02 Jan03 Jan04 Jan05 Jan06 Dec06
(b)
Plume volume (m^3 x 10^{10})
(c)
Plume centroid X coordinate in the SPCS (km)
(d)
Plume centroid Y coordinate in the SPCS (km)
Jan99 July99 Jan00 July00 Jan01 July01 Jan02 July02 Jan03 July03 Jan04 July04 Jan05 July05 Jan06 July06
Date

Fig. 6. 1999–2006 time series for the Columbia River plume metrics computed from our model simulations. While salmon survival rates from return counts for years following 2003 were not available at the time of the study, plume metrics from model simulations were generated through 2006. Model simulations for years prior to 1999 could not be generated because of the lack of consistent data sets for ocean conditions to force the model. Boxes indicate the salmon outmigration seasons (April to mid-June). Dates use two-digit years. From top to bottom: (*a*) area of the surface plume; (*b*) plume volume; (*c*) monthly distribution of the *x* coordinate of the centroid of the surface plume in the state plane coordinate system (SPCS), which represents the plume proximity to the coast (the dotted horizontal line identifies the location of the coast in the same coordinate system); (*d*) monthly distribution of the *y* coordinate of the centroid in the SPCS, representing the plume location along the north–south direction relative to the river mouth (the dotted horizontal line identifies the location of the mouth). Also shown is the transition from a negative to a positive phase of the PDO, hence from cooler to warmer ocean conditions along the Pacific coast of North America. While precipitation extremes tend to increase when PDO and ENSO are in phase (Hamlet and Lettenmaier 1999; Barton and Ramírez 2004), variability in Columbia River discharge is left unexplained. Panels (*c*) and (*d*) are modified from Burla et al. (2010).

deteriorated for the 2002 migrants. The 2003 migrants, instead, entered the ocean during a warm phase of the PDO, facing poor large-scale ocean conditions.

The positive and significant correlation we found during the cool PDO phase (1999, 2001, 2002) between anomalies in the survival rates for steelhead and anomalies in the plume size suggests that a larger plume (in particular a larger surface plume) near the time of ocean entry of juvenile steelhead favored their survival. The negative correlation with anomalies in the location of the plume suggests that a plume extending farther offshore, rather than attached to the shoreline, also led to higher steelhead survival. We interpret, instead, the poor correlations between the same anomalies in 2003, after the transition to a warm PDO phase, as a consequence of a modest contribution — in a regime of poor ocean conditions — of local (plume) conditions to the overall survival compared with the impact of large-scale ocean conditions. In years of favorable large-scale ocean conditions, the effect of local conditions becomes appreciable.

The lack of similar, consistent correlation patterns for Chinook salmon suggests that the Columbia River plume plays a different role as habitat for steelhead and Chinook. This difference is consistent with the notion that Chinook salmon, even when they enter the ocean as yearlings, reach the ocean as smaller fish than steelhead and appear to reside for a longer time in the coastal waters, closer to shore (R.L. Emmett, National Marine Fisheries Service, Northwest Fisheries Science Center, Newport, Oregon, personal communication, 2006). In contrast, looking at the distinct peaks in the correlograms for steelhead, we speculate that steelhead benefit from the plume environment as they enter the ocean, but that they mainly use the plume to move quickly away from coastal habitats and the predation pressures associated with this environment. This postulate is consistent with the finding that steelhead in particular concentrate at the plume frontal regions (De Robertis et al. 2005), whereas yearling Chinook salmon tend to be associated with the overall plume habitat when compared with the ocean (more saline) habitat. Moreover, these findings are consistent with the migration patterns of yearling Chinook salmon and steelhead we can discern from our observations. Whereas we can track yearling Chinook moving north along the coastal shelf margin from May through July, we typically observe steelhead in May, but seldom catch any in our coastal surveys after May. These findings suggest that steelhead may make a more direct and rapid migration to ocean habitats in the Gulf of Alaska, underlying the finding why plume structure may benefit their success more directly than for yearling Chinook salmon.

To what extent should the lack of a uniform response of steelhead and yearling Chinook salmon to plume structure serve as support for or against the putative relationships described herein? Although there may be a perceived notion that salmonids use essentially the same habitats, evidence suggests habitat use is species specific. Along with a general higher abundance of juvenile salmon in plume waters and frontal zones than in the surrounding, more saline ocean waters, observations have shown a differential distribution of juveniles of different species in different habitats, although with variability across years. For example, Schabetsberger et al. (2003) found yearling and subyearling Chinook salmon to be present in much higher numbers closer to shore and to be on average smaller than juvenile coho (*Oncorhynchus kisutch*), which were caught farther offshore during their study. Bi et al. (2007) confirmed these findings when evaluating a suite of physical and biological features that describe unique habitat features that differently influence the distribution and abundance of juvenile Chinook and coho salmon in coastal Pacific Northwest waters.

We further speculate that the stronger correlations of steelhead SARs with the 2001 plume volume can be explained by the much smaller plume formed by the Columbia River in that drought year. A small plume would be particularly limiting and its variability in size more critical to juvenile steelhead trying to migrate offshore. In years of higher river discharge, like 1999 and 2002, when the plume is larger, its size would be less limiting but its location relative to the coast in response to winds would become more critical (consistently with the more significant correlations of steelhead SARs with the plume proximity to the coast than with plume size in 1999 and 2002). Our results also suggest that in a year like 2001, juvenile steelhead depended upon the plume environment for a period on the order of days following ocean entry.

The fact that the conditions that steelhead encounter in the plume at the time of ocean entry can explain only part of their overall survival (16%–40% of its variability) does not surprise us. Most mortality of salmonids during their marine residence is hypothesized to occur shortly after they enter the ocean (Pearcy 1992). However, SAR is a metric that encompasses several stages in the life history of the fish and multiple years and therefore likely reflects many compounding and confounding factors (Williams et al.

2005) over this period. At this stage our goal was not to predict survival and production based upon predicted physical conditions in the plume region, rather to infer possible cause–effect relationships between variability in the plume and variability in salmon survival.

Critical uncertainties

Our results are clearly not conclusive because of a number of limitations in the analysis. First, the study used estimates of salmon survival from PIT tags. The small numbers of returning adults upon which the SARs are based make their estimate fairly imprecise, even more so when, during the migration season, the SAR ratio is computed starting from a low number of PIT-tagged juveniles. Despite the imprecision in the estimates of salmon survival, which is expressed by the wide confidence intervals shown in Appendix A, we believe that the trends of within-season variability are correctly captured by the data.

Second, variability in the actual time of ocean entry may have weakened the strength of the correlations, where they peak shortly after estimated entry time of steelhead to the ocean. This variability makes the true lag between the correlated time series not necessarily unique within each year, since the lag is a function of the time of ocean entry. Nevertheless, quantifying how much the variability in the time of ocean entry impacts the strength of the correlations is not as critical to the conclusions of this study.

Although our study could cover only 4 years of data, those years are representative of a wide range of the local-scale ocean conditions determined by the presence of the Columbia River plume (Fig. 6). Those local conditions were the focus of our investigation. The monthly Columbia River flow is only weakly correlated with the PDO (Keefer et al. 2008). During the 3 years in our study that precede the transition from a cold to a warm PDO phase, Columbia River discharge ranged from the very high flows of 1999 to the record drought of 2001, both years also characterized by La Niña conditions. ENSO-neutral conditions and highly variable flows characterized the 2002 migration season. Moderate flows, but considerably higher than in 2001, were observed in 2003, a warm PDO and El Niño year. The volume and surface area of the Columbia River plume computed from our simulations reflect this broad range of flow conditions (Fig. 6). Also in terms of proximity to the coast and south–north orientation relative to the river mouth, the plume structure varied over a wide range during the 4 years of the study. Both river flow and winds determined such variability across years as well as within each migration season. Our results were robust to the high interannual variability in local ocean (plume) conditions, until the regime shift in the large-scale ocean conditions occurred.

It must also be noted that all results from this study refer to transported fish. Given the data set used, we had no means to infer differences or similarities with survival of in-river migrants.

Alternative interpretations

Is the plume environment the true causative agent for the fraction of the total variability in the SARs found to be correlated with changes in the plume structure? Plume conditions and steelhead survival rates could both be simply responding to local upwelling, which several studies have indicated as a potential factor affecting salmon survival through bottom-up forcing of the marine food web (see Scheuerell and Williams 2005 for an example addressing this hypothesis). However, attributing to this mechanism the intraseasonal variability in steelhead survival would not explain why a similar response to increased productivity in the coastal environment was not found also in Chinook survival rates in our analysis. The utilization of the plume environment that we have postulated earlier in the discussion instead discriminates between the two species. Besides, the stratification determined by the presence of the plume may locally hinder effective upwelling (Hickey et al. 2005). In addition, during our study period there were instances (such as at the beginning of the 2002 migration season) where the strength of the anomalies in the plume metrics could not be explained by a response to upwelling-favorable winds alone, rather by a concurrent increase in river discharge. Indeed, later upwelling events, which were not accompanied by increases in river discharge, did not generate plumes of comparable characteristics and neither were they accompanied by comparably high SARs for steelhead.

Final considerations

The need to account for ocean conditions when addressing salmon management and restoration has been widely recognized (e.g., Pearcy 1992; Kareiva et al. 2000; Scheuerell and Williams 2005). In most cases researchers have pointed to the ocean as a source of variability that needs to be isolated and eliminated from analyses, to correctly assess restoration efforts and inform adaptive management experiments in the freshwater environment (Anderson 1997; Hilborn and Coronado 1997; Casillas 1999). Recognizing the ocean as a source of variability does not imply shifting the focus from the freshwater to the ocean environment for bottlenecks to salmon production and relieving management decisions upstream. On the contrary, management strategies directly guided by the knowledge of the conditions downstream (such as timing of transportation of smolts, hatchery releases, hydropower management) become available when we establish an impact for local ocean features like the Columbia River plume on Pacific salmon survival (Williams et al. 2005). By predicting conditions off the river mouth, the simulation capabilities made available by CORIE potentially provide an effective approach both to timely support those management decisions and to learn about the mechanisms governing salmon survival success in the coastal margin ecosystems. In this study, we used CORIE simulations in a correlation analysis with available biological data to formulate the hypothesis of a role for the Columbia River plume dynamics in the early marine survival of steelhead. We speculate that the lack of supportive evidence for a similar role of the plume in the early marine survival of juvenile Chinook, at least at the fine scale of variability considered in the study, is not contradictory and suggests means to explore how yearling Chinook salmon and steelhead may use these local ocean habitats differently. The hypotheses generated from this analysis should be further pursued through continuation of focused observational studies. Future studies should focus on validating the results in the context of interannual and interdecadal variability, using longer time series,

and on investigating residence time of juvenile salmon in the plume and differential distribution of species against plume structure again in the context both of intraseasonal and interannual variability.

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Appendix A

The following two tables show SARs for PIT-tagged Snake River steelhead (Table A1) and spring–summer Chinook salmon (Table A2), before filtering. 95% confidence intervals are shown. Intervals are *t*-based estimates, except for cases where there were no adult returns. For such cases (shown in italics) the upper limit of the 95% confidence intervals (CI) for the zero survival rate was estimated with the "rule of 3" (Hanley and Lippman-Hand 1983). Anomalously high values in the SARs relate to cases where the ratios were computed starting from a very low number of tagged juveniles: in such cases the return of just a few fish causes the SAR values to spike.

Table A1. SARs for PIT-tagged Snake River steelhead (*Oncorhynchus mykiss*).

	1999		2001		2002		2003	
Date	SAR (%)	95% CI	SAR (%)	95% CI	SAR (%)	95% CI	SAR (%)	95% CI
8 Apr.	0	*0–9.38*	—	—	—	—	—	—
10 Apr.	0	*0–6.12*	—	—	10	0–19.30	1.55	0–3.29
12 Apr.	0	*0–37.50*	4.05	1.11–6.99	7.77	2.60–12.94	4.35	0–9.16
14 Apr.	4.35	0–13.17	4.97	1.61–8.33	14.58	4.60–24.56	—	—
16 Apr.	0	*0–25.00*	—	—	2.56	0–6.07	0	*0–12.00*
18 Apr.	1.96	0–5.77	1.59	0–3.37	2.69	0.37–5.01	1.14	0–3.35
20 Apr.	0	*0–5.77*	2.49	0.34–4.64	2.72	1.05–4.39	3.33	0.12–6.54
22 Apr.	1.19	0–2.52	3.70	1.18–6.22	—	—	2.00	0–5.88
23 Apr.	0.90	0–1.91	—	—	1.69	0–4.98	12.77	3.23–22.31
24 Apr.	1.55	0.20–2.90	4.42	2.07–6.77	1.37	0–4.04	7.69	0–23.79
25 Apr.	1.75	0.46–3.04	—	—	0	*0–2.61*	0	*0–23.08*
26 Apr.	0	*0–1.28*	3.75	1.75–5.75	0	*0–5.56*	0	*0–50.00*
27 Apr.	2.26	0.30–4.22	—	—	3.64	0–8.59	—	—
28 Apr.	0.49	0–1.44	5.19	3.80–6.58	—	—	—	—
29 Apr.	0.55	0–1.62	3.30	1.91–4.69	—	—	3.45	0–10.39
30 Apr.	0	*0–1.72*	4.19	1.80–6.58	4.76	0–11.20	0	0–18.75
1 May	2.43	0.33–4.53	3.31	2.30–4.32	1.92	0–5.65	5.88	0–17.98
2 May	0.81	0–2.40	1.03	0.36–1.70	6.67	0–15.98	8.00	0–19.20

Table A1 (*concluded*).

	1999		2001		2002		2003	
Date	SAR (%)	95% CI	SAR (%)	95% CI	SAR (%)	95% CI	SAR (%)	95% CI
3 May	0.82	0–2.42	2.84	2.08–3.60	0	*0–6.82*	0	*0–17.65*
4 May	1.56	0–4.60	2.55	1.74–3.36	0	*0–6.38*	—	—
5 May	0.76	0–2.24	1.22	0.38–2.06	—	—	—	—
6 May	0.79	0–2.34	2.01	1.00–3.02	—	—	5.56	0–16.95
7 May	1.59	0–4.68	1.80	0.75–2.85	4.17	0–8.44	0	*0–17.65*
8 May	2.70	0–5.31	1.01	0–2.15	2.56	0–7.52	6.25	0.32–12.18
9 May	0	*0–3.30*	0.72	0.70–2.14	1.56	0–4.60	13.33	0–32.16
10 May	—	—	1.42	0.03–2.81	0	*0–8.82*	1.71	0–4.06
11 May	1.11	0–3.28	1.00	0–2.39	4.76	0–6.44	—	—
12 May	2.25	0–5.33	1.47	0–3.12	—	—	—	—
13 May	2.44	0–5.78	1.44	0–3.42	—	—	1.25	0–3.68
14 May	3.23	0.12–6.34	—	—	4.08	0–9.62	0	*0–5.26*
15 May	2.56	0–5.42	1.75	0–4.16	0	0–12.00	2.20	0–5.21
16 May	—	—	0	*0–1.92*	0	0–8.82	2.50	0–5.92
17 May	—	—	—	—	0	0–10.71	0.81	0–2.38
18 May	5.60	1.57–9.63	—	—	2.63	0–7.72	—	—
19 May	0.75	0–2.22	0.99	0.20–1.78	0	0–7.69	—	—
20 May	3.60	0.50–6.70	0.61	0–1.30	0	0–4.84	1.80	0.48–3.12
21 May	4.92	1.08–8.76	—	—	2.70	0.57–4.83	—	—
22 May	0.85	0–2.52	0.33	0–0.97	0	*0–3.37*	2.99	0.81–5.17
23 May	—	—	0.70	0.02–1.38	1.01	0–2.40	1.17	0–2.48
24 May	—	—	1.14	0–2.43	1.61	0–3.42	2.52	0.09–4.95
25 May	—	—	—	—	3.56	1.99–5.13	1.96	0–4.65
26 May	1.45	0.45–2.45	0	*0–3.45*	—	—	—	—
27 May	0.34	0–1.01	—	—	3.64	1.17–6.11	2.15	0.07–4.23
28 May	—	—	—	—	—	—	4.21	0.17–8.25
29 May	—	—	—	—	3.91	0.55–7.27	2.80	0–5.93
30 May	—	—	1.56	0–4.60	—	—	2.86	0–6.76
31 May	—	—	0	*0–6.00*	2.39	0.84–3.94	0	0–3.66
1 June	—	—	0	*0–8.57*	1.45	0.03–2.87	0	0–5.56
3 June	—	—	0	*0–7.69*	2.49	0.97–4.01	2.08	0–6.12
5 June	0	*0–3.26*	0	*0–37.50*	2.06	0.06–4.06	0	*0–3.06*
7 June	0	*0–12.50*	0	*0–23.08*	2.15	0–5.10	0	*0–3.13*
8 June	0	*0–8.33*	—	—	—	—	—	—
9 June	0	*0–18.75*	0	*0–19.5*	0	*0–9.38*	—	—
11 June	0	*0–15.00*	—	—	0	*0–8.57*	—	—

Table A2. SARs for spring–summer Chinook salmon (*Oncorhynchus tshawytscha*).

	1999		2001		2002		2003	
Date	SAR (%)	95% CI	SAR (%)	95% CI	SAR (%)	95% CI	SAR (%)	95% CI
31 Mar.	1.10	0–2.61	—	—	—	—	—	—
2 Apr.	0	*0–2.0000*	—	—	—	—	—	—
4 Apr.	3.77	0–8.90	—	—	—	—	—	—
6 Apr.	0	*0–6.25*	—	—	—	—	—	—
8 Apr.	0.89	0–2.63	—	—	—	—	—	—
10 Apr.	1.27	0–3.73	—	—	0	*0–8.57*	0.29	0–0.85
12 Apr.	0	*0–16.67*	3.66	0–7.72	1.05	0–2.50	0	*0–2.16*
14 Apr.	2.08	0–6.12	1.84	0–4.36	0.82	0–2.42	—	—
16 Apr.	6.06	0–14.20	—	—	0	*0–2.03*	0.23	0–0.68
18 Apr.	0.42	0–1.25	1.04	0–3.05	0.38	0–1.13	0.37	0–1.09
20 Apr.	0.65	0–1.92	1.48	0–3.50	1.44	0.18–2.70	0	*0–2.75*
22 Apr.	0	*0–0.52*	0	0–2.24	—	—	0.51	0–1.51
23 Apr.	0.68	0–1.44	—	—	0	*0–5.08*	0	*0–0.84*
24 Apr.	1.03	0.03–2.03	0.52	0–1.53	0	*0–4.22*	0	*0–1.18*

Table A2 (*concluded*).

	1999		2001		2002		2003	
Date	SAR (%)	95% CI	SAR (%)	95% CI	SAR (%)	95% CI	SAR (%)	95% CI
25 Apr.	3.47	1.68–5.26	—	—	1.28	0–3.04	0.36	0–1.07
26 Apr.	1.79	0.47–3.11	0.91	0–2.16	2.33	0–4.93	0	*0–2.33*
27 Apr.	1.58	0.61–2.55	—	—	1.59	0.05–3.13	—	—
28 Apr.	2.86	1.75–3.97	0.83	0.17–1.49	—	—	—	—
29 Apr.	3.50	2.00–5.00	0	*0–0.54*	—	—	0	*0–1.31*
30 Apr.	1.85	0.58–3.12	1.54	0.81–2.27	1.51	0–4.46	0	*0–3.30*
1 May	4.08	1.60–6.56	1	0.38–1.62	0	*0–8.11*	0	*0–5.66*
2 May	2.84	0.60–5.08	0	*0–0.67*	0	*0–5.00*	0	*0–2.56*
3 May	3.55	0.97–6.13	1.41	0.37–2.45	0	*0–3.90*	0	*0–2.44*
4 May	1.35	0.18–2.52	0.21	0–0.62	1.01	0–2.98	—	—
5 May	3.64	1.30–5.98	1.86	0.58–3.14	—	—	—	—
6 May	0.63	0–1.87	0.90	0.31–1.49	—	—	0	*0–2.11*
7 May	1.15	0–3.39	2.43	1.38–3.48	0	*0–4.11*	0	*0–1.55*
8 May	5.22	1.16–9.28	1.09	0.14–2.04	0	*0–9.37*	0	*0–0.93*
9 May	2.48	0.08–4.88	0.36	0–1.07	0	*0–3.33*	0	*0–1.64*
10 May	—	—	0.63	0.08–1.18	1.89	0–3.66	0	*0–2.94*
11 May	6.98	2.58–11.38	1.16	0.48–1.84	0	*0–7.14*	—	—
12 May	4.17	0–9.82	0.77	0.20–1.34	—	—	—	—
13 May	0	*0–7.32*	0.43	0–1.02	—	—	0	*0–3.26*
14 May	0	*0–7.50*	—	—	0	*0–5.77*	0	*0–2.54*
15 May	6.90	0–16.54	0.29	0.27–0.85	0	*0–6.52*	0	*0–4.48*
16 May	—	—	—	—	2.33	0–6.83	0	*0–3.80*
17 May	—	—	0.63	0.13–1.13	0	*0–10.71*	0.83	0–2.44
18 May	0	*0–6.67*	—	—	2.38	0–6.99	—	—
19 May	2.5	0–7.34	1.89	0.83–2.95	0	*0–11.11*	—	—
20 May	4	0–12.09	1.43	0.38–2.48	4.65	0–10.94	2.13	0.57–3.69
21 May	13.33	0–32.16	—	—	1.55	0–3.68	—	—
22 May	0	*0–15.00*	0.46	0–1.36	0.92	0–2.19	1.26	0–2.99
23 May	—	—	1.52	0.04–3.00	3.65	1.00–4.30	3.09	0–6.54
24 May	—	—	1.13	0.03–2.23	3.68	0.52–6.84	0	*0–2.07*
25 May	—	—	—	—	2.43	0.77–4.09	0	*0–2.31*
26 May	5.88	0.29–11.47	0.96	0–2.29	—	—	—	—
27 May	0	*0–8.33*	—	—	1.60	0–3.80	0	*0–0.66*
28 May	—	—	—	—	—	—	0	*0–2.54*
29 May	—	—	—	—	0.81	0–2.38	2.27	0–5.38
30 May	—	—	0	*0–4.00*	—	—	0.61	0–1.45
31 May	—	—	0	*0–5.26*	1.18	0–2.50	0.47	0–1.39
1 June	—	—	0	*0–6.12*	0.82	0–2.42	0	*0–1.86*
3 June	—	—	0	*0–4.92*	0.68	0–1.62	0	*0–3.80*
4 June	0	*0–30.00*	—	—	—	—	—	—
5 June	3.33	0–10.03	0	*0–5.36*	1.37	0–2.91	0	*0–2.68*
7 June	2.17	0–6.38	0	*0–0.91*	0.87	0–2.57	0.68	0–2.00
8 June	0	*0–2.61*	—	—	—	—	—	—
9 June	0	*0–9.68*	0	*0–0.50*	0	*0–9.68*	—	—
11 June	2.33	0–6.83	—	—	0	*0–75.00*	—	—

Reference

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Long-term exposure of Atlantic cod (*Gadus morhua*) to components of produced water: condition, gonad maturation, and gene expression

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Abstract: Effects related to offshore contamination have been observed in gadoid species in the North Sea, but uncertainties regarding exposure conditions have led to the need for environmentally relevant exposure studies. In the current study, Atlantic cod (*Gadus morhua*) were sampled at regular intervals during an 11-month continuous or pulsed water-borne exposure to a mixture of produced water components. There was variation in time to female maturation between and within treatment groups; although differences were not significant, there was a tendency towards delayed maturation in intermittently exposed females not seen in continuously exposed females. Physiological parameters such as condition factor, liver somatic index, gonadosomatic index, or hematocrit did not appear to be affected by the treatments. A range of toxicologically relevant genes were differentially expressed in the exposed fish, including AhR-responsive genes (CYP1A, UDP-GT) and genes relevant to immune function (complement C3, MHC 1, CYP27B), apoptosis (PERP), and oxidative stress (hepcidin, serotransferrin, glutathione peroxidase). Although powerful in their depiction of the complexity of cellular responses to environmental stressors, the use of molecular tools should be accompanied by biochemical and physiological endpoints, and results should be holistically interpreted in terms of individual health and function.

Résumé : Des effets reliés à la contamination au large ont pu être observés chez des espèces de gadoïdés dans la mer du Nord, mais les incertitudes associées aux conditions d'exposition rendent nécessaires des études sur l'exposition qui soient pertinentes au niveau de l'environnement. Dans notre étude, nous avons prélevé des morues franches (*Gadus morhua*) à intervalles réguliers durant une exposition continue ou intermittente en milieu aqueux à un mélange de composantes de l'eau produite (par les forages pétroliers). Au sein des groupes expérimentaux et d'un groupe à un autre, il y avait des variations dans le temps requis pour atteindre la maturation chez les femelles; bien que les différences ne fussent pas significatives, il y avait une tendance vers une maturation retardée chez les femelles exposées par intermittence, mais pas chez les femelles exposées de manière continue. Les variables physiologiques, telles que le coefficient de condition, l'indice hépatosomatique, l'indice gonadosomatique et l'hématocrite, ne semblent pas avoir été affectées par les traitements. Une variété de gènes d'importance toxicologique étaient exprimés de diverses manières chez les poissons exposés, tels que les gènes réagissant à AhR (CYP1A, UDP-GT) et les gènes pertinents pour la fonction immunitaire (complément C3, MHC 1, CYP27B), l'apoptose (PERP) et le stress oxydatif (hepcidine, sérotransferrine, glutathion-peroxydase). Bien que puissants dans leur dépiction de la complexité des réponses cellulaires aux facteurs de stress de l'environnement, les outils moléculaires ne devraient être utilisés que conjointement avec des données d'analyse biochimique et physiologique et les résultats devraient être interprétés de manière holistique dans le contexte de la santé et du fonctionnement des individus.

[Traduit par la Rédaction]

Introduction

Large volume discharges of produced water to sea from offshore oil and gas production platforms have led to concerns for impacts on North Sea and, more recently, Barents Sea fish stocks. Produced water contains several inorganic and organic contaminants including polycyclic aromatic hydrocarbons (PAHs) and alkylphenols (APs) (Utvik 1999), compounds that have been associated with adverse effects in fish such as DNA damage and cancer development (Myers et al. 2003), oxidative stress (Sturve et al. 2006), developmental toxicity (Incardona et al. 2006), immune system modulation (Reynaud and Deschaux 2006), and impacts on reproductive processes (Harris et al. 2001; Barse et al. 2006; Meier et al. 2007*b*). The volume of discharged produced water in the Norwegian sector alone was 162 million m^3 in 2007 (The Norwegian Oil Industry Association 2008). Because of rapid dilution following discharge to

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sea, environmental concentrations of PAHs and APs are in the nanograms per litre ($ng \cdot L^{-1}$) range even close to produced water discharge points (Durell et al. 2006; Utvik and Gärtner 2006), making the assessment of biological impact challenging. There is an extensive knowledge of how PAHs may affect fish following short-term exposure to high concentrations (Beyer et al. 1997; Willett et al. 2001; Incardona et al. 2005), and recent studies with Atlantic cod (*Gadus morhua*) have clearly indicated that exposure to APs through the diet may affect vital processes in this species (Meier et al. 2007*a*, 2007*b*). The major exposure route of produced water components for marine fish species will be through water, and although some studies have assessed short-term and (or) high concentration exposures (Martin-Skilton et al. 2006; Sturve et al. 2006; Abrahamson et al. 2008), there are only a few published studies of long-term exposure to environmentally relevant concentrations of PAHs and APs (e.g., Grung et al. 2009; Holth et al. 2009).

Atlantic cod is a geographically widespread and, despite recent population declines in some areas, economically important species in the northern Atlantic. Atlantic cod has been the species of choice for Norwegian coastal and offshore monitoring programs for the last couple of decades (Ruus et al. 2003; Hylland et al. 2008, 2009) and was used as the model species in caging studies during a recent international workshop, Biological Effects of Contaminants in Marine Pelagic Ecosystems (Hylland et al. 2006*b*). The results from the workshop indicated that cod caged for 5–6 weeks at different distances from the platform were exposed (Aas et al. 2006) and to some extent affected by the effluent as measured in glutathione *S*-transferase (GST) activity (Danischewski 2006) and plasma vitellogenin concentration (Scott et al. 2006*b*). There was no change in other markers for sublethal effects (e.g., peroxisomal proliferation) (Bilbao et al. 2006*a*), DNA adduct formation (Balk et al. 2006), and hepatic cytochrome P4501A concentration and activity (Förlin and Hylland 2006). The environmental relevance of caging studies can be questioned because of the brevity of the exposure and the varying availability of food (but see Hylland et al. (2004) and Oikari (2006)). Although results have not been consistent, there is some evidence that other gadiids have been affected by offshore-related contamination in the North Sea; e.g., lysosomal membrane stability, peroxisomal proliferation, and parasite prevalence in saithe (i.e., pollock, *Pollachius virens*) (Bilbao et al. 2006*b*) and GST activity, phase I system components, and DNA adduct formation in haddock (*Melanogrammus aeglefinus*) (Hylland et al. 2006*a*).

During environmental monitoring, uncertainties related to exposure duration, concentration, or chemical composition would be substantial owing to fish movement and varying levels of exposure. Also, the wide range of known and unknown environmental contaminants and physical factors has made the isolation of contaminant-related effects problematic. Therefore, there is a need for well-designed, long-term exposure studies to aid in the assessment of possible impacts from environmentally relevant concentrations of contaminants.

In recent years, efforts to sequence stress-specific genes in cod have provided cDNA libraries including clones to be used in toxicogenomic transcriptome analysis (Bowman and Trippel 2005; Olsvik 2005). In acute as well as chronic exposure studies, characterization of differentially expressed genes could help in identifying potential mechanisms of observed effects on higher levels of biological organisation. Unfortunately, causal links between effects at different levels of biological organisation are still poorly understood. Often, contaminant concentrations in exposure studies are above environmentally relevant levels to provoke an effect. This may provide valuable mechanistic information on the potential effects of contaminants, but threshold levels of effect or contaminant interactions may complicate their extrapolation to a field situation.

The aim of the current study was to clarify whether long-term (11 month) exposure to environmentally relevant concentrations of components of produced water would affect sublethal responses and (or) population-relevant endpoints in Atlantic cod. In previous publications on this experiment, we describe bile metabolites (Grung et al. 2009), contaminant concentrations in water and their relationship to bile metabolites (Harman et al. 2009*a*), and genotoxicity (Holth et al. 2009). Fish were sampled at regular intervals throughout the exposure period from each of four experimental groups (control, continuous low, pulsed high, continuous high). Samples from individual fish were analysed for physiological parameters and gene expression. The gonad maturation of cod from each of the experimental groups was assessed at the end of the exposure period.

Materials and methods

Experimental

Juvenile farmed cod, weight 490.6 g ± 3.4 g and length 35.6 cm ± 0.3 cm (average ± standard error, SE), expected to be first time spawners at the end of the experiment period, were distributed into control (four replicate tanks) and three treatment groups (each threereplicate tanks); 73 fish per tank. Tank volumes were 1 m^3. All fish were weighed, measured, and tagged individually using passive integrated transponder (PIT) tags, then distributed in appropriate sex ratios based on vitellogenin measurements. The fish were acclimated to a flow-through exposure system for 30 days before start of the exposure. The treatment groups comprised environmentally relevant concentrations (continuous low, continuous high, and pulsed high) of low-molecular-weight PAHs (four or fewer rings), short-chained APs (four or fewer carbons in alkyl chain), and phenol, combined in a cocktail representative of produced water from the North Sea (Table 1). The continuous high and low concentration treatment groups received a total of 5.4 and 0.54 $\mu g \cdot L^{-1}$ PAHs and 11.4 and 1.14 $\mu g \cdot L^{-1}$ APs, respectively. The pulsed treatment group received alternatively high and control exposures at 2-week intervals. Acetone was used as carrier, also included in the control group exposure (6 $\mu L \cdot L^{-1}$). The nominal contaminant water concentrations were confirmed by the use of semipermeable membrane devices for the first 4 weeks of the experiment (Harman et al. 2009*a*). Because of the low exposure concentrations, special adaptations were made to the design to separate any contaminant-specific effects from interfering effects such as variation in physiochemical water parameters, carry-over contamination of volatile compounds, and adsorption of exposure chemicals or contaminants in feed. The water intake was at 60 m

Table 1. Nominal concentrations of the polycyclic aromatic hydrocarbons (PAHs) and alkylphenols (APs) used in experiment.

Type	Group	Compound	High exposure concentration ($ng \cdot L^{-1}$)	Low exposure concentration ($ng \cdot L^{-1}$)
Phenol	Phenol	Phenol	7000	700
AP	C1-phenol	*p*-cresol	7500	750
	C2-phenol	4-ethylphenol	3150	315
	C3-phenol	4-*n*-propylphenol	615	62
	C4-phenol	4-*tert*-butylphenol	175	18
PAH	Naphthalene	Naphthalene	1550	155
	Acenaphtylene	Acenaphthylene	0.025	0.003
	Acenaphthene	Acenaphthene	9	0.9
	Fluorene	Fluorene	62	6
	Anthracene	Anthracene	4	0.4
	Phenanthrene	Phenanthrene	80	8
	Dibenzothiophene	Dibenzothiophene	19	2
	Pyrene	Pyrene	6	0.6
Alkylated PAH	C1-naphthalene	2-methylnaphthalene	1700	170
	C2-naphthalene	2,6-dimethylnaphthalene	945	95
	C3-naphthalene	2-ethyl-6-methylnaphtalene	565	57
	C1-phenanthrene	9-methylphenanthrene	108	11
	C1-anthracene	2-methylanthracene	100	10
	C2-anthracene	9,10-dimethylanthracene	100	20
	C2-phenanthrene	9-ethylphenanthrene	119	12
	C3-phenanthrene	9-*n*-propylphenanthrene	18	2
	C1-dibenzothiophene	4-methyldibenzothiophene	23	2
	C2-dibenzothiophene	4-ethyldibenzothiophene	33	3

Note: Pulsed exposure alternated every second week between high exposure and control treatment. Adapted from Holth et al. (2008) and Grung et al. (2009).

depth in the outer Oslofjord, with an average temperature and salinity of 8.4 °C ± 1.2 °C and 34.2 ± 0.4 psu, respectively, throughout the exposure period. The fish were kept at natural photoperiod (May–March). To prevent carry-over of volatile chemicals, air circulation between tanks was limited using plastic curtains. Chemical adsorption was minimized by coating or lining tubings, pumps, and header tanks with polytetrafluoroethylene (PTFE). The fish were fed daily (0.5% *w/w* biomass) with Barents Sea white fish fillet (not cod) for the first 2 months, then with specially designed pellets (Nutreco, Amersfoort, Netherlands) prepared from essential nutrients and specially purified fish oil (Pronova Biopharma Norge AS, Oslo, Norway).

Chemicals and equipment

PAHs and APs were purchased from Chiron AS (Trondheim, Norway) and Sigma-Aldrich (St. Louis, Oregon, USA). Acetone was purchased from Merck KGaA (Darmstadt, Germany). The purity of all compounds was at least 98% analytical grade. Acetone stock solutions of selected PAHs and APs were kept at –20 °C prior to use. Header tanks and tubings were made of or lined with PTFE. Metering pumps, lined with PTFE, were purchased from Iwaki Norge AS (Kolbotn, Norway).

Sampling procedure and gross parameters

Prior to each sampling, the fish were starved for 2 days to ensure accumulation of bile. From each tank, three fish were terminated at time 0 (prestart). After 2 and 4 weeks of exposure, eight fish from each tank were terminated. After 8, 16, 32, and 44 weeks of exposure, a minimum of six fish was terminated per tank. The fish were randomly selected, but sampled to achieve an even sex ratio. Each fish was killed by a blow to the head, and a blood sample was taken from the caudal vein using a syringe treated with a combination of heparin (Sigma-Aldrich) and aprotinin (Sigma-Aldrich). Hematocrit was measured using hematocrit capillaries (Sigma-Aldrich) and a hematocrit centrifuge (Compur M1100, Bayer Diagnostics). Length and weight were registered, and bile was withdrawn using a syringe. The bile was frozen at –20 °C prior to PAH metabolite measurements (cf. Grung et al. 2009). The liver was weighed, split into replicates, and frozen on liquid nitrogen. After 44 weeks of exposure, total weight of gonads was determined and gonad samples (1–2 g) were immediately fixed in more than 20 mL 3.6% buffered formaldehyde (29.5 $mmol \cdot L^{-1}$ NaH_2PO_4 and 460 $mmol \cdot L^{-1}$ Na_2HPO_4) for determination of oocyte diameter. Also, a random selection of male and female individuals from each treatment was monitored for another 4 weeks and, if running, stripped for assessment of in vivo fertility. Although the sex of the fish could not be exclusively determined on external characteristics, the sex ratio of the selection was approximately 1:1 based on measured plasma vitellogenin concentrations before start of the experiment (fish distributed by PIT tags).

Physiological endpoints

Fulton's condition factor (K) was calculated as (body weight/length^3) × 100. Liver somatic index (LSI) and gona-

dosomatic index (GSI) were calculated as organ to body weight percentages. Hematocrit was determined as percent (*v/v*) red blood cells. After 44 weeks of exposure, the mean diameter of 200 oocytes was estimated by the autodiametric fecundity method, and potential fecundity was calculated using the formula $F_p = 2.139 \times 10^{11} \times OD^{-2.709}$ (µm) × OW (g), where OD is oocyte diameter and OW is ovary weight (Thorsen and Kjesbu 2001). Relative potential fecundity was calculated as F_p/body weight. Leading cohort diameter was calculated as the average of the 10% largest oocytes in the sample, and days to start of spawning (y) as $y = 3.33 \times 10^6 \times d^{-1.817}$, where d is the leading cohort diameter (Kjesbu 1994).

Microarray strategy and RNA isolation

Hepatic RNA was extracted from two males and two females from each tank after 4 and 32 weeks of exposure. Individuals exposed to PAH and AP were compared with a pool of eight corresponding control fish using a two-colour system cDNA microarray, the CodStress array (Lie et al. 2009*a*). In this work, the second generation version of the CodStress array was used, consisting of 1056 clones. The control pools, unique for each sex and time, were made by mixing an equal amount of total RNA from all individuals. Dye-swap experiments were performed using a total of six slides and six biological replicates for each experimental group (Dabney and Storey 2007).

Extraction of total RNA was performed according to the Trizol manufacturer's protocol (Invitrogen, Carlsbad, California). Liver tissue was thawed on ice, resuspended in 1 mL of Trizol reagent, and homogenized twice at 4 °C, 23 Hz for 30 s using 5 mm tungsten carbide grinding beads (Retsch GmbH, Haan, Germany) and an MM301 homogenizer (Retsch GmbH, Haan, Germany). To prevent co-precipitation of proteoglycans, the precipitation of RNA using isopropanol was replaced with 1:1 *v/v* isopropanol:precipitation buffer (8.9 mmol·L^{-1} disodium hydrogencitrate; 12 mmol·L^{-1} NaCl). RNA was further purified and DNase treated using EZNA Total RNA Kit I (Omega Bio-tek, Georgia) including a DNase set RNase-free (Omega Bio-tek). The samples were eluted in 80 µL of diethylpyrocarbonate-treated RNase-free water and aliquoted in duplicates for microarray and RT-qPCR analyses. RNA yield was determined using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, USA). RNA integrity was determined by capillary electrophoresis using a 2100 Bioanalyzer instrument (Agilent Technologies, Santa Clara, California). None of the samples used for microarray showed signs of degradation or impurities (260/280 and 260/230 >1.8, RNA integrity number >8.0).

Target labelling and hybridization

Total RNA (10 µg) was used for cDNA synthesis by the Superscript Indirect cDNA kit (Invitrogen, Carlsbad, California). The cDNA was labelled with either Cy3-dUTP (Amersham, Buckinghamshire, UK) or Cy5-dUTP (Amersham) and purified on S.N.A.P. columns using the cDNA labelling purification module (Invitrogen). The incorporation efficiency was examined by measuring the cDNA concentration at 260 nm, Cy3 incorporation at 550 nm, and Cy5 at 650 nm using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies). Eighty nanograms of each labelled cDNA target were mixed and denatured at 100 °C for 2 min before addition of 11 µL blocking agent (Agilent Technologies) and 55 µL 2× GE× hybridisation buffer HI-RPM (Agilent Technologies). The final volume was adjusted to 110 µL with DNase free water. Microarray slides were prehybridized at 65 °C for 60 min using 1% bovine serum albumin (BSA) Fraction V, 3.5× saline sodium citrate (SSC) and 0.1% sodium dodecyl sulfate (SDS). Hybridization was performed at 62 °C for 16 h using Agilent gasket slides, hybridization chamber, and oven. Gasket slides were removed in 2× SSC and 0.1% SDS at 65 °C before they were twice washed for 5 min in 1× SSC and 0.2× SSC, respectively (first wash at 65 °C, second wash at room temperature). Finally, slides were washed for 45 s in 0.05× SSC at room temperature and dried immediately by centrifugation.

Microarray scanning and data processing

Microarray slides were scanned using a DNA Microarray Scanner (Agilent Technologies). The photomultiplier tube voltage used was 30% of maximum voltage. Generated pictures were split, flipped, and imported into the Genepix software (Molecular Devices, Sunnyvale, California) for grid alignment and extraction of raw signals. Extracted data have been deposited in the ArrayExpress database (www.ebi.ac.uk/arrayexpress, accession No. E-MTAB-121). Extracted raw data were imported into J-Express Pro V 2.7 (Molmine AS, Bergen, Norway) and corrected for background (Smyth 2004). All flagged and control spots were removed previous to within (global Loess method) and between (scale method) array normalizations (Smyth and Speed 2003). Low-intensity spots were then filtered (FG – BG < 1.5 × BG SD). In-array replicates were combined using median values. Genes with more than 75% missing values were filtered, and the least square impute adaptive method was used for the remaining missing indices (Bø et al. 2004). The final gene expression matrix was split into subsets for sex, treatment, and time, allowing a maximum of 25% estimated missing values.

Reverse transcription-qPCR (RT-qPCR)

Transcriptional levels of eight differentially expressed genes and one unregulated gene (microarray data) were quantified using RT-qPCR, in the same total RNA samples as in the corresponding microarray experiment. Also, three genes not included on the array (vitellogenin a, nm23, zona pellucida 3) were quantified. Vitellogenin is considered a biomarker of exposure to compounds acting through the estrogen receptor (ER) (Emmersen and Petersen 1976). Also, zona pellucida 3 as well as a nucleoside diphosphate kinase (nm23) are responsive to estrogen treatment in several fish species (Oppen-Berntsen et al. 1992; Gunnarsson et al. 2007).

Three reference genes (ubiquitin, S9, ARP-2) were included in addition to no-template and no-RT controls. A standard curve (1000–31.25 ng, twofold dilutions) was made in triplicates for control of reaction efficiency for each gene.

The RT-qPCR steps were performed as described in Olsvik et al. (2009), except final concentration of each dNTP in the RT reaction was 500 µmol·L^{-1}, and concentrations of

forward and reverse primers in qPCR were both 250 nmol·L^{-1}. PCR primers were designed with amplicon size <145 base pairs close to the 3′ end using Primer Express 2.0 software (Applied Biosystems, Foster City, California) and purchased from Invitrogen (primer sequences provided in Supplemental Table S1[3]).

Statistical analyses

Statistical analyses for weight, length, condition factor, liversomatic index, gonadosomatic index, hematocrit, and fecundity were carried out by a two-way analysis of variance with interaction (restricted maximum likelihood method) (Sokal and Rohlf 1994), using treatment and exposure time as fixed effects. Replicate tanks for each treatment were included as random factors and nested in treatments. For all parameters, the treatments compared were control, low, pulse, and high. The number of fish sampled from each tank was three before start of the experiment, eight after 2 and 4 weeks of exposure, and six at all other samplings. As far as possible, the fish were sampled to achieve an even sex ratio. The χ^2 test was used to test for differences in numbers of spawning fish (Sokal and Rohlf 1994).

Before analyses of microarray data, redundant probes were collapsed to genes (max probe). Rank product analysis (Breitling et al. 2004), as implemented in J-Express Pro V 2.7 (Molmine AS), was used on each expression subset to identify genes consistently up- or down-regulated. Genes with a false discovery rate < 0.05 were regarded as significantly differentially expressed (400 permutations), and subset lists combined to produce a final differential gene expression matrix. Another rank product analysis was used on the matrix to identify the most consistent significant gene regulations in the data set.

qPCR reference gene stability was evaluated using the *geNorm* tool. This tool determines the most stable genes from a set of tested genes and calculates a gene expression normalization factor for each sample based on the geometric mean of a user-defined number of reference genes (Vandesompele et al. 2002). The two most stable reference genes (ubiquitin and S9) were used for normalization. Differential expression between control and treated samples was analysed using the Relative Expression Software Tool (REST) 2008 (Pfaffl et al. 2002). The test was iterated 2000 times, and $\alpha < 0.05$ was considered significant.

Results

Bile metabolites

PAH and AP metabolites were determined in bile of male and female cod exposed for 4, 8, 16, 32, and 44 weeks by high-performance liquid chromatography (1-OH-phenanthrene and 1-OH-pyrene) and gas chromatography – mass spectrometry (time of flight) analyses and reflected exposure conditions well as previously described (Grung et al. 2009; Holth et al. 2009). In summary, a concentration-dependent relationship was observed for most metabolites at all time points, although the relationship was less clear for the more volatile compounds. The concentration of metabolites in bile rose with increasing compound lipophilicity (Grung et al. 2009). For most metabolites, the concentration ratio in bile between the continuously high and low exposure groups was around 10, similar to the nominal concentration ratios in water between the same treatments. The concentration of 1-OH-pyrene in the continuously low exposed group was in accordance with concentrations observed in fish caged near oil installations in the North Sea (Aas et al. 2006). The metabolite levels in the fish receiving pulsed high exposures were similar to the levels observed in the continuously high exposed fish at all samplings

Physiological endpoints

Male or female weight, length, condition (K), LSI, GSI, or hematocrit appeared not to be affected by the treatments (for all parameters: df = 3, $p > 0.05$) (male and female data in Supplemental Tables S2 and S3, respectively[3]). For all parameters, significant changes over time were observed. A 40% decrease in male LSI (df = 6, F ratio = 16.8, $p < 0.0001$) and 50% decrease in female LSI (df = 6, F ratio = 35.3, $p < 0.0001$) were observed throughout the experiment (as illustrated in Figs. 1 and 2, respectively). Male and female GSI decreased between weeks 0 and 4 and remained low until week 16, after which the values increased (male: df = 6, F ratio = 6.8, $p < 0.0001$; female: df = 6, F ratio = 6.7, $p < 0.0001$). Male and female condition factor decreased for the first 4 weeks (male: df = 6, F ratio = 34.5, $p < 0.0001$; female: df = 6, F ratio = 20.2, $p < 0.0001$), but then remained stable throughout the experiment. Significant differences over time were also observed in male (df = 5, F ratio = 4.93, $p < 0.0003$) and female (df = 5, F ratio = 4.84, $p < 0.0003$) hematocrit levels. The mortality during the experiment was 2%–3% per month, as reported in Holth et al. (2009).

Gonad maturation

Follicle diameter was determined and potential fecundity and time to start of spawning estimated to evaluate maturation stage in all sampled females after 44 weeks of exposure (Table 2). There were no significant differences between the treatments (all parameters: df = 2, $p > 0.05$). The variation between tanks within treatment was substantial, especially in the control group, accounting for 20%–40% of the total variation in the model for most parameters. All values of leading cohort oocyte diameter were lower, and the calculated days to spawning were higher in the pulsed high group compared with the females exposed in the continuous high group. In the continuous low exposure group, only one of the sampled fish had reached the vitellogenic stage, and this treatment had to be omitted from statistical analyses.

Starting from week 44, a random selection of males and females was monitored for an additional 4 weeks and if running, stripped for assessment of reproductive output as well as F_1 generation effects (Table 2). The number of females successfully stripped (in vivo) was not significantly different following statistical analyses (χ^2 test). Assessment of fertilization success, larval mortality, hatching time, and development was performed by hatching eggs and incubating larvae until depletion of the yolk, but because of the low number of simultaneously spawning fish in the exposed groups, comparisons could not be made.

[3] Supplementary data for this article are available on the journal Web site (http://cjfas.nrc.ca).

Fig. 1. Development of somatic parameters in male Atlantic cod through the course of the experiment: (*a*) liversomatic index (LSI), (*b*) gonadosomatic index (GSI), (*c*) condition factor (*K*), and (*d*) hematocrit. Data are presented as median and range of control ($n = 4$, solid circles and solid line), low ($n = 3$, open circles and solid line), pulsed ($n = 3$, solid squares and dashed line), and high dosed group ($n = 3$, open squares and dotted line).

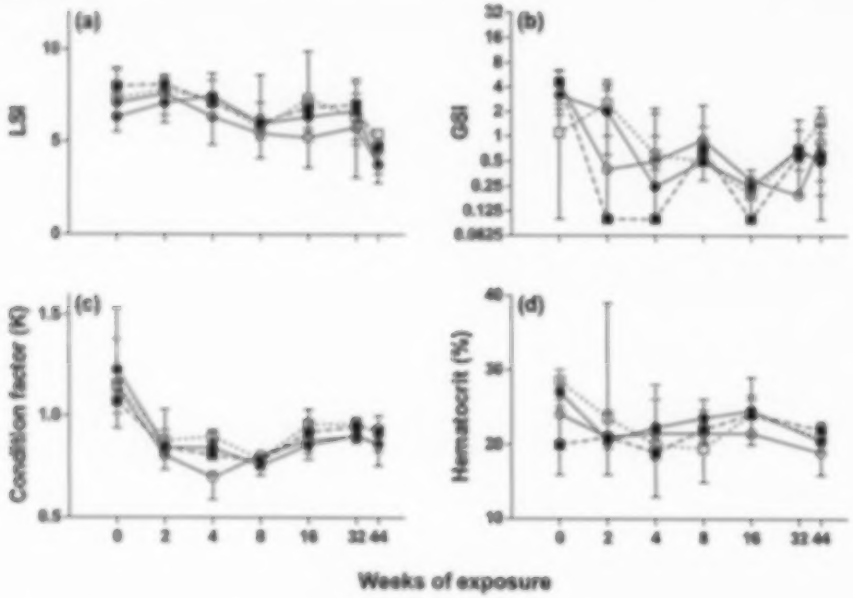
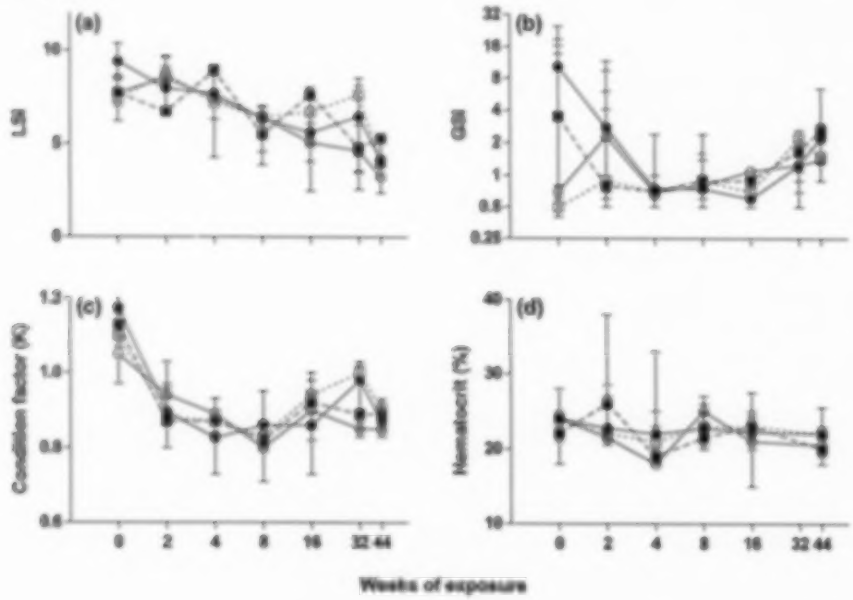


Fig. 2. Development of somatic parameters in female Atlantic cod through the course of the experiment: (*a*) liversomatic index (LSI), (*b*) gonadosomatic index (GSI), (*c*) condition factor (*K*), and (*d*) hematocrit. Data are presented as median and range of control ($n = 4$, solid circles and solid line), low ($n = 3$, open circles and solid line), pulsed ($n = 3$, solid squares and dashed line), and high dosed group ($n = 3$, open squares and dotted line).



mRNA transcript expression

The overall difference of mRNA ratios between control and treated fish as detected with microarrays was moderate, with a maximum fold-change ratio between control and treated groups of 2.8 (serotransferrin). A subset of eight differentially expressed genes was chosen for RT-qPCR analysis, in addition to aryl hydrocarbon receptor 2, a gene not apparently differentially regulated according to the microar-

Table 2. Fecundity parameters estimated by autodiametric fecundity method (median).

	Control				Low		Pulse			High		
Variable	A	B	C	D	A	B	A	B	C	A	B	C
No. of previtellogenic females	4	7	6	3	4	5	6	4	5	5	5	6
No. of vitellogenic females	4	1	2	1	0	0	2	4	0	0	1	3
No. of spawning females	1	0	2	0	1	0	0	1	1	2	2	0
Follicle diameter (μm)	498	367	578	315	768	NA	336	359	334	539	595	397
Potential fecundity (× 1000)	872	1387	794	908	NA	NA	976	1545	830	1120	777	1334
Potential relative fecundity	1053	1534	1047	1335	NA	NA	1285	1616	1109	1213	869	1390
Leading cohort diameter (μm)	607	439	708	364	1162	NA	426	446	384	715	686	486
Time to spawning (days)	29.2	52.6	22.1	73.9	9.0	NA	35.6	52.7	67.1	22.0	23.4	43.7
No. of fish*	8	8	7	6	6	5	8	6	6	8	7	8
No. of spawning males*	1	2	1	1	1	1	0	2	0	1	2	1
No. of spawning females*	2	2	0	2	0	1	0	1	1	1	1	1

Note: There were no significant differences between treatments. The fecundity calculations were based on mean vitellogenic oocyte diameter of 200 oocytes per fish (diameter: $500 < x < 850$ μm). Presence of ovulated eggs or hydrating oocytes were used as spawning markers.
*In vivo.

ray results. Overall, the RT-qPCR results corresponded well with the microarray data in female fish. In male fish, there was a lack of correspondence between some RT-qPCR results and microarray data for plasminogen, caspase 8, and for the continuous low concentration group, CYP1A. This might be due to a lack of control group variation caused by pooling of tissue in the microarray experiment (control samples were not pooled for RT-qPCR) or to differences in microarray and PCR probe–primer sequence specificity or sensitivity.

Twenty-five differentially expressed mRNA transcripts were identified from the gene expression matrix in males (Table 3) and females (Table 4) by rank product analysis ($q < 0.05$), some of which have been known to respond to xenobiotic exposure, e.g., CYP1A, UGT, and glutathione peroxidase (complete matrix of differentially expressed genes (110 genes) provided in Supplemental Table S4[3]). A dose-related response was observed in CYP1A mRNA levels in both males and females, although a reduction (RT-qPCR) was observed in the continuous low concentration group males. The UGT mRNA levels corresponded to the CYP1A levels for some of the groups, but appeared to be overall less responsive.

Two genes related to metabolism and transport of iron (hepcidin, serotransferrin) were up-regulated in females and in some of the male groups. A similar, but less distinct pattern was observed for the regulation of glutathione peroxidase (GPX4), which was up-regulated in females after 4 weeks of exposure. In microarray analyses of males, GPX4 appeared up-regulated in the pulsed high and continuous high concentration groups, but this finding was not confirmed by RT-qPCR.

Some genes related to regulation of the cell cycle and apoptosis (PERP, caspase-8, cell division cycle 14) were differentially expressed, of which PERP was down-regulated in all but the continuous high exposure groups after 32 weeks of exposure. Immune system related genes such as MHC class 1 and complement C3 was up-regulated in some groups, more consistently in females than in males. The immune system related genes Fc fragment of IgG binding protein and interleukin-8 were down-regulated in the same groups.

Genes related to general metabolism of endogenous and exogenous compounds were CYP4B, CYP27B, and glutamine synthetase. CYP4B was up-regulated in all groups, whereas CYP27B and glutamine synthetase were among the most down-regulated genes in all groups. Also, some genes considered as housekeeping genes (glyceraldehyde 3-phosphate dehydrogenase, elongation factor 1 alpha, eukaryotic translation initiation factor 3) were up-regulated in all groups. Differentially expressed genes of unknown toxicological relevance were cathepsin S, heat shock factor 2, plasminogen, 14 kDa apolipoprotein, and TFIIA P12 subunit (transcription factor 2a).

In addition, the mRNA levels of three genes (nm23, VTGa, ZP3) not present on the microarray were quantified (RT-qPCR). The nm23 mRNA level was 1.5 times up-regulated ($p < 0.05$) in female cod from the pulsed high group after 4 weeks of exposure compared with the control group. In male cod, there was a down-regulation (–2.0) of ZP3 mRNA levels in the pulsed high group after 4 weeks of exposure, in addition to up-regulations in the pulsed high (1.5) and continuous high (1.7) groups after 32 weeks of exposure. Cp values (number of PCR cycles required to exceed background levels) for VTGa mRNA transcript levels in most male and female cod were above 33 and often close to or equal to 40 (below detection limit), indicating very low levels of VTGa mRNA. Levels of VTGb mRNA were not measured.

Discussion

In the current study, juvenile prespawning Atlantic cod were exposed to three different treatments of low-molecular-weight polycyclic aromatic hydrocarbons (PAHs) and short-chained alkylphenols (APs) at concentrations and in a combination relevant to environmental exposure to effluents from offshore oil platforms (Utvik 1999; Harman et al. 2009*b*). Exposure conditions were monitored in water and confirmed nominal exposure concentrations for most PAHs (Harman et al. 2009*a*). PAH and AP metabolite concentrations in bile provided an excellent reflection of individual exposure to the compounds, with a ratio around 10 between the high and low treatment groups (cf. Grung et

Table 3. Hepatic gene expression ratios in males between exposed and control groups analysed by microarray (M) and RT-qPCR (P).

	4 weeks of exposure						32 weeks of exposure					
	Low		Pulsed high		High		Low		Pulsed high		High	
Name	M	P	M	P	M	P	M	P	M	P	M	P
Plasminogen	1.3	1.2	**1.5***	1.1	1.4	1.0	**2.3***	0.9	**1.6***	0.9	**1.6***	1.1
Alpha-1-antiproteinase-like protein	**1.5***		1.4		**1.6***		**1.7***		**1.5***		1.4	
Glyceraldehyde 3-phosphate dehydrogenase	**1.6***		**1.6***		**1.8***		**1.8***		1.3		1.3	
Cell division cycle 14	**1.5***		**1.8***		1.5		**1.6***		1.3		1.5	
Serotransferrin	1.3	0.8	**1.4***	1.2	1.4	1.0	**1.9***	1.6	**1.5***	**1.4**	1.3	0.9
Elongation factor 1 alpha	1.4		**1.7***		**1.5***		**1.6***		1.4		1.5	
Cytochrome P4501A	1.2	**0.6**	1.4	1.1	**1.6***	1.5	1.3	**0.2**	**1.5***	**2.5***	1.5	**3.1***
Hepcidin	1.4		1.4		1.5		**1.7***		1.3		1.1	
Saxitoxin binding protein 1	1.3		1.3		**1.5***		1.5		**1.6***		1.2	
Eukaryotic translation initiation factor 3	1.5		**1.5***		1.3		1.5		**1.4***		1.4	
UDP-glucuronosyltransferase	1.4	**0.7**	1.4	1.2	1.1	1.1	**1.5***	1.1	**1.4***	**1.4**	1.4	0.9
Caspase 8	1.4	0.8	1.3	**0.7**	1.1	1.0	1.2	1.0	**1.5***	1.3	**1.6***	0.8
Cytochrome P4504B	1.4	1.1	**1.6***	1.2	1.3	1.2	1.3	**0.5***	1.3	1.0	1.3	1.0
Complement C3	1.1	0.6	**1.9***	1.2	1.3	1.3	1.5	0.9	1.2	0.9	1.0	0.9
14 kDa apolipoprotein	1.0		1.3		1.5		1.3		**1.6***		1.3	
Phospholipid hydroperoxide glutathione peroxidase A	1.1	1.0	**1.6***	1.1	**1.4***	1.0	1.2	0.5	**1.6***	1.0	**1.4***	1.2
MHC class I	1.1		1.2		**1.7***		1.4		1.3		1.2	
Aryl hydrocarbon receptor 2	1.0	0.9	0.9	1.1	1.0	1.0	1.0	0.8	1.0	0.8	1.0	0.9
Heat shock factor 2	0.9		**0.7***		**0.6***		0.8		**0.7***		0.9	
Fc fragment of IgG binding protein	0.8		0.8		**0.7***		0.9		0.8		0.9	
Interleukin-8 variant 5	0.8		0.7		**0.7***		0.9		0.8		0.9	
PERP TP53 apoptosis effector	0.8		0.8		**0.6***		0.8		**0.7***		1.0	
Cathepsin S	0.8		0.7		**0.6***		0.9		0.8		0.9	
Glutamine synthetase	**0.7***		1.0		**0.7***		**0.5***		**0.6***		**0.6***	
TFIIA P12 subunit	**0.8***		**0.7***		**0.6***		0.7		**0.6***		0.9	
Cytochrome P45027B	**0.6**	0.5	0.9	1.9	**0.7***	1.0	**0.5***	**0.1***	**0.6***	0.3	0.6	0.2

Note: Significant up- or down-regulation have been indicated by bold text and asterisks (microarray: $q < 0.05$ rank product ($n = 3$ per treatment), RT-qPCR: $p < 0.05$ REST (same samples as analysed by microarray)). Ratios with $p < 0.1$ are indicated by bold text only.

al. 2009). The PAH metabolite concentrations in cod bile from the continuous low group were similar to concentrations observed in cod caged near offshore oil installations (Aas et al. 2006).

Effects on reproduction have previously been observed in Atlantic cod exposed to C_6–C_7 APs via feed (Meier et al. 2007*b*). In that study, exposed Atlantic cod had reduced estrogen levels, and there was interference with gonad maturation and delayed estimated time to spawning in exposed individuals. In a study by Lie et al. (2009*b*), oil-related effects were reported as lowered GSI and reduced levels of circulating plasma vitellogenin. In the current study, the size of oocytes was not statistically different between treatments. There were, however, indications of reduced oocyte diameter and increased time to spawning in the pulsed high group compared with the continuous high group, suggesting that exposure history is relevant. This apparent difference in oocyte diameter could indicate a potential for adaptation in continuously exposed individuals. Earlier studies have suggested that continuous exposure models might underestimate the risk from intermittent exposures (Butcher et al. 2006).

In the above-mentioned studies, the relationships between dose and response were unclear, suggesting that there could be a threshold level for effects of AP exposure on reproduction. In the study by Meier et al. (2007*b*), low doses produced the same decrease in 17β-estradiol or 11-ketotestosterone levels as higher doses. In the study by Lie et al. (2009*b*), there were even more pronounced differences in GSI and vitellogenin in fish exposed to low AP concentrations than in fish exposed to high concentrations. If contaminant exposure at one time more or less permanently affects systemic components of the reproductive axis, such as the hypothalamus–pituitary–gonad axis (e.g., Meier et al. 2007*b*; Zohar et al. 2010), effects could accumulate over years as cod are iteroparous batch spawners (Murua and Saborido-Rey 2003).

Induced vitellogenin levels have been observed in Atlantic cod collected in the North Sea, indicating that levels of estrogens in the open sea may affect wild fish (Scott et al. 2006*a*). In addition, a gradient of increasing plasma vitellogenin was observed towards an oil platform during a research workshop (Scott et al. 2006*b*). In addition to nonyl- and octyl-phenols, short-chained APs present in produced water, i.e., 4-*tert*-butylphenol, are known to be estrogenic (Barse et al. 2006; Tollefsen et al. 2006; Tollefsen and Nilsen 2008). To complicate the issue, some PAHs are known to be anti-estrogenic either through receptor crosstalk (Harper et al. 2006) or through inhibition of steroidogenesis (Monteiro et al. 2000). Such interactions could provide an alternative explanation for the lack of differential mRNA ex-

Table 4. Hepatic gene expression ratios in females between exposed and control groups analysed by microarray (M) and RT-qPCR (P).

	4 weeks of exposure				32 weeks of exposure			
	Low	Pulsed high		High	Low	Pulsed high		High
Name	M	M	P	M	M	M	P	M
Plasminogen	**1.8***	**2.0***	1.6	**2.5***	**1.5***	**1.8***	1.6	1.4
Alpha-1-antiproteinase-like protein	**1.7***	**1.7***		**2.5***	**1.6***	**1.7***		1.4
Glyceraldehyde 3-phosphate dehydrogenase	**1.7***	**1.5***		**2.2***	**1.6***	**1.6***		1.4
Cell division cycle 14	**1.6***	**1.8***		**2.1***	**1.5***	**1.6***		1.4
Serotransferrin	**1.7***	**1.8***	2.6	**2.8***	**1.7***	**1.6***	**1.9**	1.4
Elongation factor 1 alpha	1.2	1.3		1.4	**1.6***	**1.5***		**1.7***
Cytochrome P4501A	**1.5***	1.3	2.5	**2.1***	1.4	**1.5***	1.7	**1.7***
Hepcidin	1.4	**1.5***		1.7	**1.5***	**1.6***		1.5
Saxitoxin binding protein 1	**1.7***	1.4		**2.3***	1.2	1.3		1.2
Eukaryotic translation initiation factor 3	1.2	1.3		1.4	1.4	**1.5***		1.5
UDP-glucuronosyltransferase	1.3	1.4	**1.9***	1.2	**1.5***	**1.7***	1.6	1.5
Caspase 8	1.4	1.3	1.0	1.2	1.4	**1.7***	0.7	1.4
Cytochrome P4504B	**1.5***	1.4	1.2	1.4	**1.5***	**1.5***	1.0	1.5
Complement C3	**1.6***	**1.4***	1.6	1.3	1.2	**2.0***	**1.9**	1.1
14 kDa apolipoprotein	**1.7***	**1.5***		**2.4***	1.2	1.1		1.1
Phospholipid hydroperoxide glutathione peroxidase A	**1.8***	1.4	1.4	1.5	1.1	0.7	0.6	1.0
MHC class I	**1.7***	**1.6***		1.5	1.2	1.3		1.2
Aryl hydrocarbon receptor 2	0.9	0.9	2.0	1.0	1.0	1.0	1.5	1.0
Heat shock factor 2	**0.8***	**0.7***		**0.7***	0.8	0.9		1.0
Fc fragment of IgG binding protein	0.8	**0.8***		**0.6***	0.8	0.9		0.9
Interleukin-8 variant 5	**0.8***	**0.7***		**0.6***	0.9	0.8		0.9
PERP TP53 apoptosis effector	**0.7***	**0.7***		**0.6***	0.8	0.8		1.0
Cathepsin S	**0.8***	0.8		**0.7***	0.9	0.8		0.9
Glutamine synthetase	0.7	0.8		**0.4***	**0.6***	**0.4***		**0.5***
TFIIA P12 subunit	**0.6***	**0.6***		**0.5***	0.8	**0.7***		0.9
Cytochrome P45027B	**0.6***	**0.7***	0.6	**0.4***	**0.8***	**0.7***	1.1	**0.6***

Note: Significant up- or down-regulation have been indicated by bold text and asterisks (microarray: $q < 0.05$ rank product ($n = 3$ per treatment), RT-qPCR: $p < 0.05$ REST (same samples as analysed by microarray)). Ratios with $p < 0.1$ are indicated by bold text only.

pression of estrogen receptors (ER) or ER-mediated genes in the current study. Hepatic vitellogenin mRNA levels were generally below the detection limit and no concurrent regulation of nm23 or ZP3 genes could be observed, although an up-regulation of ZP3 mRNA in male fish exposed to the pulsed high treatment after 32 weeks suggested an estrogenic influence. In addition, the large differences in estrogen receptor agonist potential of produced water effluents has led to speculations that other compounds than APs may be responsible for the ER agonist activity of produced water (Thomas et al. 2009).

The reduction in male and female LSI was not related to exposures, but indicated utilization of lipid reserves. This was presumably due to an increased lipid demand during gonad maturation. The increase in female GSI from week 16 indicated the start of maturation as the fish where approaching the spawning period. During an annual reproductive cycle, considerable changes in both LSI and GSI would be expected (Hylland et al. 1998), as energy is allocated to gonad growth and reproduction. Therefore, the observed changes in LSI and GSI could be considered as part of the normal reproduction cycle in Atlantic cod. Other gross parameters such as condition factor and hematocrit also appeared to be unaffected by the treatments. Both condition factor as well as hematocrit are considered general indicators of fish health and may be affected by various stress factors (Mayer et al. 1992; Ziskowski et al. 2008). Hematocrit does not appear to be a sensitive parameter for exposure to water-soluble oil components (Kennedy and Farrell 2005). A reduction in condition factor with corresponding effects on genes related to lipid metabolism has previously been observed in zebrafish (*Danio rerio*) exposed to a similar treatment (Holth et al. 2008). Similarly, depressed growth was observed for turbot (*Psetta maximus*) exposed to low concentrations of produced water (Stephens et al. 2000). Although gross parameters may be useful in assessing general stress in several fish species, other factors appeared to outweigh eventual exposure related effects in the current study.

A decline in condition factor was observed during the first 2 weeks of the experiment, which could indicate a general stress response. Although the fish were adapted for a long period to the exposure system, initiation of exposure required a more intense sampling scheme than later in the experiment. Following this period, fish were again growing significantly. This condition factor decline might have been a response to the increased sampling rate. An alternative explanation might have been the introduction of the carrier solvent (acetone; 6 $\mu L \cdot L^{-1}$) into the experiment, although the concentration was well below recommended maximum concentration for chronic studies (Hutchinson et al. 2006).

Several differentially expressed gene transcripts related to stress were identified by rank product analysis. Cytochrome P4501A (CYP1A) and UDP-glucuronosyl transferase (UGT) represented responses from the aryl hydrocarbon receptor (AhR)-battery of genes (Köhle and Bock 2007). The concentration related response in CYP1A mRNA levels indicated the presence of AhR agonists in the exposure mixture. In females, CYP1A mRNA was up-regulated in all groups, but without an apparent relationship to CYP1A activity (determined as EROD) (Holth et al. 2009). Also, the concentration of CYP1A protein (measured by ELISA, results not shown) was at the same level in all groups, which may indicate alterations of translational processes or decreased protein stability. For long-lived proteins, discrepancies between mRNA and protein levels might be expected (Kloepper-Sams and Stegeman 1994). Also, poor correspondence among CYP1A mRNA, protein levels, and activity has earlier been observed in common dab (*Limanda limanda*) (Kammann et al. 2008) and flounder (*Platichthys flesus*) (George et al. 2004) collected in polluted areas. CYP1A mRNA ratios were constant or increasing over time, suggesting a lack of adaptation, at least at the gene expression level, during the 32 weeks of exposure. The regulation of the phase II enzyme UGT appeared to be less responsive and related to CYP1A expression. Up-regulation of UGT and CYP1A mRNA have previously been observed in flounder collected in a PAH and metal polluted area (Williams et al. 2003).

Effects on iron metabolism were clearly indicated by up-regulations of hepcidin and serotransferrin. The free iron content may catalyze ROS generation and oxidative stress (Livingstone 2001). Hepcidin, a key mediator of iron metabolism (Harrison-Findik et al. 2006), has previously been found to be up-regulated in response to oxidative stress in fish hepatocytes (Finne et al. 2007). Also, the regulation of hepcidin was responsive both after exposure to a model inducer of oxidative stress (paraquat) and after exposure to a mixture of toxicants (EE2, NQO, PQ, TCDD) (Finne et al. 2007). mRNA transcripts of the iron transporter serotransferrin have been observed up-regulated in flounder after exposure to several pesticides (Marchand et al. 2006) and polluted sediments (PAH, heavy metals) (Williams et al. 2003). Serotransferrin may cause prooxidant activity (Klebanoff and Waltersdorph 1990) as well as activation of the immune system (Bayne et al. 2001). Similarly, in the current study, hepcidin as well as serotransferrin appear sensitive and responsive in fish following exposure to components of produced water. The expression of phospholipid hydroperoxide glutathione peroxidase (GPX4) also appeared to be affected, further indicating an oxidative stress response. Glutathione peroxidase has been suggested as a useful biomarker for both hypoxia and hyperoxia stress in liver of Atlantic cod (Olsvik et al. 2006) and is considered to play an important role in the protection of membranes from lipid peroxidation (van der Oost et al. 2003). Increased levels of DNA adducts were observed in the same fish in the high exposure group late in the exposure period (Holth et al. 2009).

The exposure to PAHs and APs also had effects on the transcription of components of the immune system, such as up-regulation of MHC class I and complement C3 in females. Effects of PAHs on the immune system of fish are well known (Reynaud and Deschaux 2006) and have previously been observed in cod after exposure to APs (Lie et al. 2009*b*) as well as in zebrafish following exposure to low concentrations of PAHs and APs (Holth et al. 2008). Complement C3 has central functions in the immune system (Holland and Lambris 2002) and may also play a role in apoptosis and homeostasis in cod (Lange et al. 2005). In addition to genes directly involved in the immune response, genes related to apoptosis, such as PERP, were affected. In stressed cells, PERP has a proapoptotic role, whereas loss of PERP function may lead to loss of responsiveness to apoptotic signals (Nowak et al. 2005). The down-regulation of PERP could therefore lead to further modulation of immune responses. Another gene possibly affecting the immune system was CYP27B, the most consistently down-regulated gene transcript. The enzyme encoded by this gene is critical in the synthesis of vitamin D3 (Kim et al. 2007), regulating calcium homeostasis (Larsson et al. 2002). Recently, effects of vitamin D3 on the immune system have been demonstrated in gilthead seabream (*Sparus aurata*) (Cerezuela et al. 2009). It is not clear whether modulation of immune responses could pose a threat to fish populations, although a relationship between contaminant levels and disease prevalence has been observed in common dab collected from the North Sea (Hylland et al. 2006*a*).

In conclusion, exposure was confirmed by a concentration-dependent relationship between nominal PAH and AP water concentrations and their respective metabolite concentrations in bile. There were indications that exposure to pulsed high concentrations of PAHs and APs may increase the time to start of spawning in Atlantic cod, whereas general health parameters (condition, LSI, GSI, and hematocrit) appeared to be largely unaffected by any of the treatments over a period of 11 months. Expression of gene transcripts related to the activation of the AhR pathway was affected by the exposures, as well as gene transcription related to immune function, apoptosis, and oxidative stress. Exposure to the elevated concentrations of PAHs and APs did not appear to strongly affect estrogen-regulated gene expression.

In this study there were no clear gene expression signals for subsequent reproductive modulation (delayed gonad maturation). We believe that long-term studies such as that reported here are necessary to increase our understanding of a development of responses in fish subject to chronic low-level contaminant exposure such as that found in many coastal areas. While gene expression studies are useful for their ability to display the complexity of a response to contaminants, there is a clear need for biochemical and particularly physiological parameters in such studies. Rather than simply using molecular methods to predict higher-level responses, they should be seen as an additional component in understanding the total response of the individual to contaminant stress.

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Limited diversity in natal origins of immature anadromous fish during ocean residency

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Abstract: Variable migration patterns can play a major role in promoting diverse life history traits among populations. However, population and stage-specific movement patterns are generally unknown yet crucial aspects of life history strategies in many highly migratory species. We used a natural tag approach using geochemical signatures in otoliths to identify natal origins of 1-year-old anadromous American shad (*Alosa sapidissima*) during ocean residency. Otolith signatures of migrants were compared with a database of baseline signatures from 20 source populations throughout their spawning range. Samples were dominated by fish from only two rivers, while all other potential source populations were nearly or completely absent. These data support the hypothesis that American shad exhibit diverse migratory behaviors, and immature individuals from populations throughout the native range do not all mix on northern summer feeding grounds. Rather, our results suggest populations of anadromous fish are distributed heterogeneously at sea in the first year of life and thus may encounter different ocean conditions at a critical early life history stage.

Résumé : Les patrons variables de migration peuvent jouer un rôle significatif en favorisant une diversité des traits du cycle biologique chez les populations. Cependant, les patrons de déplacement spécifiques à la population et aux différents stades sont généralement inconnus, bien qu'ils soient des aspects essentiels des stratégies du cycle biologique chez plusieurs espèces fortement migratrices. Nous avons utilisé une méthodologie de marquage naturel en identifiant par les signatures géochimiques des otolithes les sites de naissance d'aloses savoureuses (*Alosa sapidissima*) anadromes de 1 an durant leur résidence en mer. Les signatures des otolithes des migrateurs ont été comparées à une banque de signatures caractéristiques de 20 populations sources réparties sur l'ensemble de l'aire de reproduction. Les échantillons sont dominés par des poissons provenant de seulement deux rivières, alors que toutes les autres populations sources potentielles sont presque ou entièrement absentes. Ces données appuient l'hypothèse qui veut que les aloses savoureuses possèdent des comportements migrateurs diversifiés et que les individus immatures des populations sur l'ensemble de l'aire de répartition naturelle ne s'entremêlent pas tous sur les sites nordiques d'alimentation d'été. Au contraire, nos données indiquent que les populations de poissons anadromes se répartissent en mer de manière hétérogène durant leur première année de vie et qu'elles peuvent donc faire face à des conditions océaniques différentes à ce stade précoce critique de leur cycle biologique.

[Traduit par la Rédaction]

Introduction

Migratory behavior is a taxonomically widespread phenomenon that allows individuals to exploit diverse habitats and resources over the course of their lifetime (Dingle 2006). These movements are often seasonally timed to ensure individuals move to those regions more favorable to survival and growth (Dingle 1996; Roff 2002). Migratory routes thus reflect behavioral decisions to optimize trade-offs between energetic costs and mortality risks associated with traveling long distances and the fitness benefits of moving to a potentially more favorable habitat (Baker 1978; Dodson 1997). These trade-offs have direct consequences on future reproductive performance and can substantially influence life history traits, including semelparity (Bell 1980; Hutchings 1993). While variation in migratory behavior is common (Ketterson and Nolan 1983; Quinn and Brodeur 1991), the degree to which individuals from each population follow common migratory routes at a given time is often unknown. However, life history characteristics such as mean growth rates, maturation schedules, and reproductive output among populations often vary and migratory behaviors may be similarly diverse, reflecting population-specific requirements and trade-offs. Such variable behavior may expose individuals from different populations to divergent environmental conditions during their migratory movements, with important implications for growth and survival (Metcalfe 2006). Alternatively, populations that consistently follow common migratory routes may exhibit differential survival in poor ocean conditions, leading to selective mor-

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tality. Understanding population-specific migratory behavior and geographic distributions at a given life history stage is necessary to assess the interplay between environmental conditions and the future performance of each population.

A basic problem when studying migratory species is the identification of natal origins (Webster et al. 2002). This apparently straightforward question can be difficult to answer in practice, particularly for species that travel long distances and breed in numerous discrete locations (Rubenstein and Hobson 2004). Artificial tagging of adults on spawning grounds cannot directly identify natal origins at the time of recapture without assuming natal homing of tagged individuals. Mitochondrial and nuclear DNA markers are only useful in identifying natal origins for species with significant variability in allele frequencies among populations at the spatial scale of interest. A promising alternative approach to determining natal origins employs the chemical composition of tissues as a natural tag (Hobson 1999). The method requires geographic variability in a chemical signature, such as an isotope ratio, that is reliably incorporated into the tissue. Migratory patterns of birds and butterflies have been successfully studied using natural gradients in hydrogen, carbon, and nitrogen isotope ratios that are recorded in feather and wing keratin (e.g., Hobson et al. 1999; Rubenstein et al. 2002). The chemistry of fish ear bones (otoliths) has been particularly useful in tracking movement patterns because otoliths are metabolically inert, continuously accrete material in successive layers, and their chemical composition reflects to some degree that of ambient water at the time of material deposition (Campana 1999; Bath et al. 2000; Walther and Thorrold 2006). Thus if a chemically distinct natal habitat signature exists, it is recorded in the core of an otolith for life, allowing natal origins and movement patterns since birth to be determined retrospectively (Thorrold et al. 2001). In particular, natural geochemical signatures in otoliths can reveal the movements of fish during their early life history stages, in which relatively small fluctuations in survival and growth can have considerable influence on subsequent population dynamics (Sogard 1997).

Little is known about population-specific distributions of many ocean-phase anadromous species. Most work has focused on identifying origins of Atlantic (*Salmo salar*) and Pacific (*Oncorhynchus* spp.) salmon using a variety of intrinsic and extrinsic markers. Artificial tags, genetic markers, and otolith microstructure and chemical signatures have been used successfully to identifying high rates of philopatry (Quinn 1993), distinguish hatchery from wild individuals (Barnett-Johnson et al. 2008), and to estimate mixed-stock compositions of oceanic fisheries (Seeb et al. 2004; Beacham et al. 2006). Recently, analyses of coded wire tag returns and DNA markers have revealed intriguing information about stock-specific distributions of juvenile and immature Pacific salmonids (Trudel et al. 2009; Tucker et al. 2009; Weitkamp 2010). This work demonstrated that individuals were more likely to be captured near their natal river during the first year of ocean residency and that recapture distances increased with age. However, the degree to which similar stock- and age-specific migration patterns apply to nonsalmonid anadromous fishes has rarely been addressed.

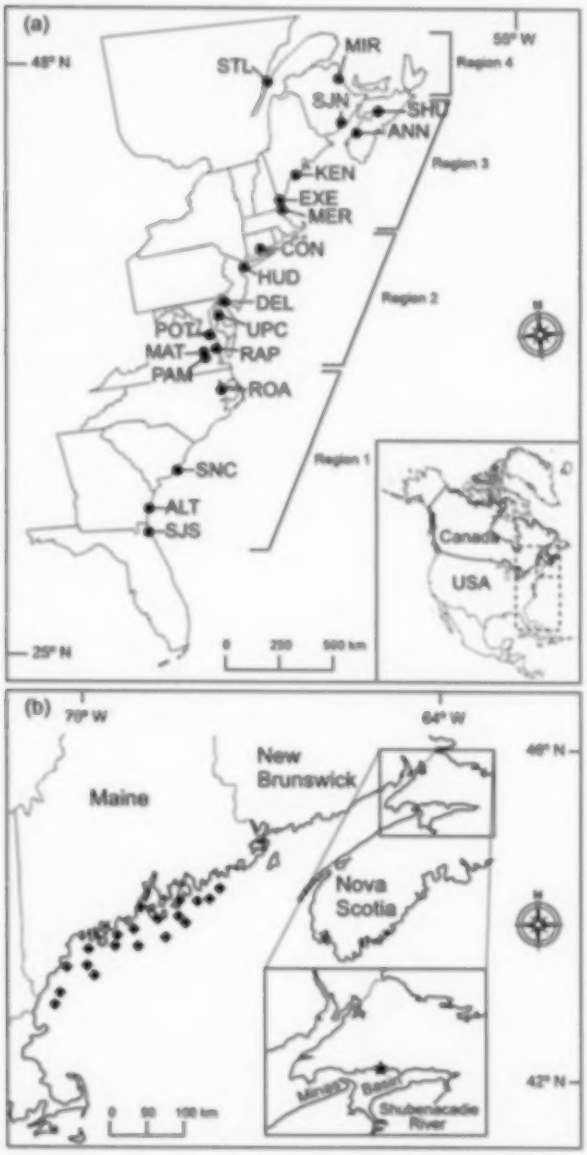
We used natural geochemical signatures in otoliths of American shad *Alosa sapidissima* to determine natal origins of immature migrants in the marine environment. American shad are anadromous alosine clupeids, spawning in freshwater habitats from Florida, USA, to Quebec, Canada (Limburg et al. 2003). These populations exhibit latitudinal gradients in life history characteristics such as growth rates and fecundity (Leggett and Carscadden 1978; Limburg et al. 2003). Most information on oceanic distributions and migration patterns of American shad populations has come from extensive mark–recapture studies on adult individuals (Talbot and Sykes 1958; Leggett 1977; Dadswell et al. 1987). Adults from all populations are thought to follow common migratory routes in the marine environment, with substantial mixing of populations on their summer feeding grounds in the Bay of Fundy (Dadswell et al. 1987). Marine movements of immature fish are less well known. While some individuals remain near their natal estuary after freshwater emigration (Hoffman et al. 2008), others move rapidly into fully marine habitats (Limburg 2001) and have been found at locations across the continental shelf during their first year (Neves and Depres 1979). Previous efforts to determine stock composition of coastal migrants used both traditional tagging methods (Dadswell et al. 1987) and population genetic analysis (Brown et al. 1999). Both approaches analyzed stock compositions of adults, and origins of immature individuals after their emigration from fresh water have not been previously identified. We thus employed geochemical tagging approaches to identify origins of immature American shad captured in coastal habitats in the Gulf of Maine to determine the diversity of sources present in these harvests.

Materials and methods

Otolith collections and analyses

American shad were collected in the spring along the coast of Maine, USA, and in the summer in Minas Basin in the Bay of Fundy, Nova Scotia, in 2005 (Fig. 1). Fish were collected in the spring by trawl surveys along coastal regions of Maine that took place from 3 May to 7 June, using a modified shrimp net with a 1 inch (1 inch = 2.5 cm) stretch mesh liner that was deployed according to a stratified random sampling design (Sherman et al. 2005). We retained all fish between 100–180 mm fork length to restrict subsequent analyses to individuals from the 2004 year class (Leim 1924; Dadswell et al. 1984). Of the 104 completed tows, American shad of the specified size range were present in 20 tows, and all individuals from these tows were retained for analyses. Catches averaged 5 fish per trawl date except for one large collection of 77 fish on 12 May (total $n = 220$). We also collected fish of the same size range at a commercial herring weir on the northern shore of Minas Basin, Nova Scotia, during low tides from June through early August. These collections spanned the entire time during which American shad of any size were present in weir catches, and all individuals in the specified size range were retained. Catches in the weir averaged 10 fish per day, with the largest collection of 66 fish occurring on 22 July (total $n = 238$). Sagittal otoliths were dissected, mounted, and ground to the midplane with fine-grained lapping film. Because all hatchery-produced American shad are marked with fluorescent oxytetracycline prior to release (Hendricks et al.

Fig. 1. (*a*) Map of rivers where otoliths and water samples were collected to parameterize source-specific multivariate otolith signatures, as described by Walther and Thorrold (2008). Abbreviated river codes follow those given in Table 1. Rivers are grouped into regions following Dadswell et al. (1987). (*b*) Collection locations of American shad sampled in 2004. Trawl surveys (diamonds) collected fish along the coast of Maine in the spring. The largest trawl collection (gray circle) of 77 fish occurred on 12 May. Summer collections were made at a weir (star) in Minas Basin in the Bay of Fundy.



1991), we examined sectioned otoliths under a UV light source for hatchery marks. No marked fish were detected.

One sagittal otolith from each pair was randomly chosen for laser ablation analysis and cleaned in a class 100 clean room. We used a Thermo Finnigan Neptune multiple collector inductively coupled plasma mass spectrometer coupled to a 213 nm laser ablation system to ablate a 250 μm × 250 μm raster adjacent to the nucleus of each otolith. This raster placement allowed us to isolate the freshwater residency period while also avoiding any maternally derived material accreted at the core. Methods included blank and mass bias corrections as outlined in Walther et al. (2008) with the following modifications. During each ablation pass, the instrument cycled among three sets of monitored isotopes: (*i*) ^{83}Kr, ^{84}Sr, ^{85}Rb, ^{86}Sr, ^{87}Sr, and ^{88}Sr were monitored simultaneously for 3 s, (*ii*) ^{48}Ca was monitored for 1 s, and (*iii*) ^{138}Ba was monitored for 1 s. By cycling through the sets of monitored isotopes, we quantified ratios of Sr:Ca, Ba:Ca, and ^{87}Sr:^{86}Sr with a single ablated raster on the core of each otolith. External precisions (relative standard deviations) based on repeated measurements of a dissolved otolith certified reference material (CRM; Yoshinaga et al. 2000) were 1.8% for Sr:Ca and 1.5% for Ba:Ca (n = 96). The isotope ratio ^{87}Sr:^{86}Sr was calculated by correcting for interferences of ^{87}Rb on ^{87}Sr and ^{86}Kr on ^{86}Sr intensities, and mass bias corrections were applied (Barnett-Johnson et al. 2005; Jackson and Hart 2006). All data were normalized to a CRM SRM987 ^{87}Sr:^{86}Sr value of 0.71024 based on mean ^{87}Sr:^{86}Sr values measured in SRM987. The mean (±1 standard deviation, SD) value of ^{87}Sr:^{86}Sr values in the SRM987 (n = 40) run throughout the analyses was 0.71026 ± 0.00003, which is within 1 SD of the true value of SRM987 (0.71024). Periodic measurements of an aragonitic marine sclerosponge (n = 9) yielded a mean (±1 SD) value of 0.70916 ± 0.00001 that was close to the global marine ^{87}Sr:^{86}Sr value of 0.70918.

The second otolith from each fish was analyzed for δ^{18}O ratios using isotope ratio monitoring mass spectrometry. A computer-controlled micromill removed a 400 μm × 400 μm raster in the same region ablated on the first otolith. Samples were analyzed on a Thermo Finnigan MAT253 equipped with a Kiel III carbonate device following methods outlined by Ostermann and Curry (2000). The long-term precision estimate of the mass spectrometer based on analyses of the NBS19 standard is ± 0.07‰ for δ^{18}O (Ostermann and Curry 2000).

Statistical analyses

Geochemical signatures in otoliths of 1-year-old migrants were compared with signatures from known-origin juveniles and water samples collected the previous year from 20 rivers along the Atlantic coast (Walther and Thorrold 2008). River-specific signatures based on Sr:Ca, Ba:Ca, δ^{18}O, and ^{87}Sr:^{86}Sr ratios were highly distinct, with an average cross-validated classification accuracy of 93%. The 20 stocks in the juvenile database represent the majority of extant spawning biomass, including all stocks that are most likely to be present in the mixed sample.

We visualized differences in otolith δ^{18}O, ^{87}Sr:^{86}Sr, Sr:Ca, and Ba:Ca ratios by plotting signatures in multivariate space using canonical discriminate plots (Fig. 2). Signatures from otoliths of 1-year-old migrants captured in the Maine trawl surveys and Minas Basin were plotted over those from known-origin juveniles (Fig. 2). The majority of core signatures from immature migrants were within the ranges of val-

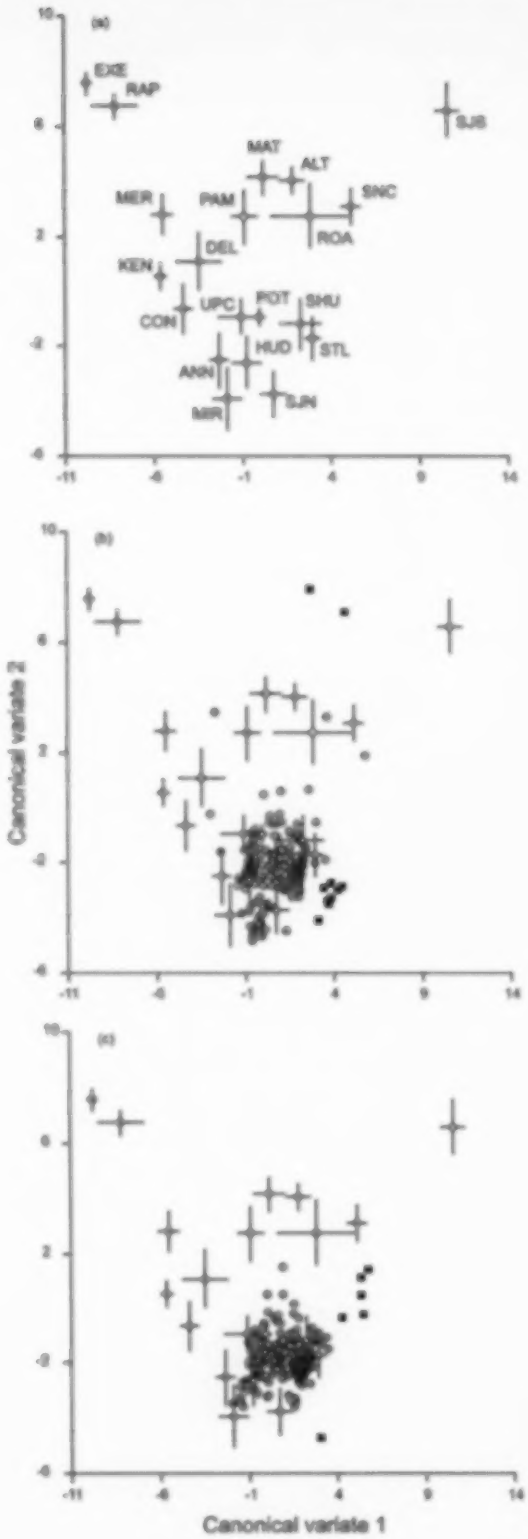


Fig. 2. (*a*) Canonical discriminant plot of isotope (^{87}Sr:^{86}Sr and $\delta^{18}O$) and elemental (Sr:Ca and Ba:Ca) signatures from juvenile otoliths or water samples collected from 20 rivers in 2004 (Walther and Thorrold 2006). Open diamonds are means (±1 standard deviation, SD) of canonical scores for each river. Abbreviated river codes follow those given in Table 1. (*b*) Canonical discriminant plot of natal geochemical signatures from otoliths in individual immature fish (shaded circles) collected in spring Maine trawl surveys along with mean (±1 SD) canonical scores for each source river (open diamonds). Solid squares are otolith signatures from fish that were excluded from composition analyses. (*c*) Canonical discriminant plot of natal geochemical signatures from otoliths of individual immature fish (shaded circles) from summer Minas Basin weir collections along with mean (±1 SD) canonical scores for each source river (open diamonds). Solid squares are otolith signatures from fish that were excluded from composition analyses.

ues defined by the ground-truthed juvenile signatures. A few signatures fell outside these ranges and likely originated from sources not included in the juvenile database. These fish were excluded from estimates of migrant stock compositions to reduce potential estimation biases. These unclassified fish represented only 5% (n = 11) and 2.5% (n = 6) of spring and summer collections, respectively. Untransformed data were used in all analyses, as normal probability plots showed only moderate departures from normality, and transformations did not alter residual distributions. Stock compositions were analyzed separately for fish collected from the spring Maine trawl surveys (n = 209) and the summer weir collections in Minas Basin (n = 232).

Stock compositions were assessed using two different types of assignment methods. For all assignment methods we used $\delta^{18}O$, ^{87}Sr:^{86}Sr, Sr:Ca, and Ba:Ca ratios for each fish as a multivariate signature. The first assignment method was the maximum-likelihood estimation (MLE) program Integrated Stock Mixture Analysis (ISMA) (Campana et al. 1999). This algorithm estimates proportions of unknown mixtures that derive from stocks parameterized by a reference data set and does not identify origins of individual fish (Millar 1987). The ISMA program was used because it allowed inclusion of all 20 potential source rivers, unlike an alternative MLE program HISEA (Millar 1990), which allows only a maximum of eight contributing sources. However, the ISMA program does not estimate variance around mixture proportions. To explore the magnitude of variance around mixture proportions, we restricted our contributing sources to the six most abundant estimated sources (St. Lawrence, Shubenacadie, St. John, Hudson, and Potomac rivers). Using these six sources as a baseline, we calculated stock mixtures and associated SDs for the summer samples using the HISEA program in bootstrap mode by resampling the mixed stock data 1000 times with replacement.

The second method was a Bayesian estimator explicitly formulated for use with continuous multivariate otolith chemistry data (Munch and Clarke 2008). For this analysis we set prior mixing proportions equal to a nearly uniform Dirichlet distribution and retained 5000 samples after discarding the initial 5000 draws. We assigned natal origins to individual fish based on the maximum posterior classification probability and then calculated proportional representations of each source stock in the two samples.

Table 1. Percent composition from each source river in mixtures of immature migrant American shad from spring Maine trawl surveys and summer Minas Basin weir collections as estimated by both the maximum likelihood estimation (MLE) and Bayesian methods.

			Spring		Summer	
River	Code	Region	MLE	Bayesian	MLE	Bayesian
Miramichi	MIR	4	—	—	—	—
St. Lawrence	STL	4	0.3	—	0.3	—
Shubenacadie	SHU	3	41.3	49.8	56.4	74.1
St. John	SJN	3	5.4	5.7	0.3	0.4
Annapolis	ANN	3	—	—	—	—
Kennebec	KEN	3	—	—	—	—
Exeter	EXE	3	—	—	—	—
Merrimack	MER	3	0.5	—	—	—
Connecticut	CON	2	0.1	—	—	—
Hudson	HUD	2	44.2	41.6	25.4	24.1
Delaware	DEL	2	—	—	—	—
Upper Chesapeake	UPC	2	1.7	1.0	—	0.4
Potomac	POT	2	5.6	-	17.7	0.9
Rappahannock	RAP	2	—	—	—	—
Mattaponi	MAT	2	—	—	—	—
Pamunkey	PAM	2	—	1.0	—	—
Roanoke	ROA	1	—	1.0	—	—
Santee-Cooper	SNC	1	1.0	—	—	—
Altamaha	ALT	1	—	—	—	—
St. Johns	SJS	1	—	—	—	—
n			209	209	232	232

Note: Recapture regions as defined by Dadswell et al. (1987) are indicated. Rivers are ordered from north (top) to south (bottom).

Results

Natural geochemical signatures in otoliths revealed that more than 97% of 1-year-old American shad in both spring and summer collections came from one of three rivers (Table 1). Based on results from the Bayesian estimator, nearly 50% of the fish from spring collections in the Gulf of Maine came from the Shubenacadie River, almost 42% were spawned in the Hudson River, and nearly 6% were from the St. John River. Fish from origins south of the Chesapeake Bay were present in negligible proportions according to both estimation methods. The Shubenacadie River was even more over-represented in the summer samples from the Bay of Fundy, accounting for just over 74% of the 1-year-old American shad. Hudson River fish represented a further ~24%, while all remaining rivers, if present, contributed less than 1% to the pooled summer sample.

There was general agreement in proportional abundances of source stocks in both collections as estimated by the MLE and Bayesian algorithms. The one exception was for the summer samples, where the MLE method suggested that 17% (approximately 40 individuals) of the fish were from the Potomac River. The Bayesian estimator did not detect significant numbers of Potomac River fish and instead classified those individuals as originating from the Shubenacadie River. This result likely arose because of similarities in chemical signatures between the Potomac and Shubenacadie rivers (Fig. 2).

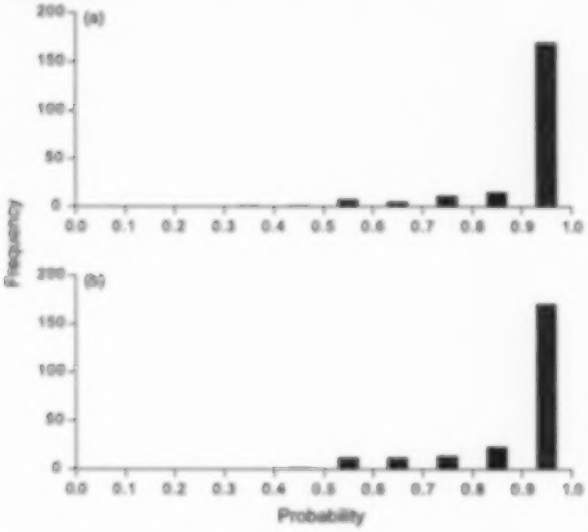
Maximum posterior classification probabilities from the Bayesian estimator were higher than 0.80 for 88% and 83% of fish collected from Maine and Minas Basin, respectively, indicating that the majority of fish were assigned to a source river with high degrees of confidence (Fig. 3). Because of the discrepancy in assignment estimates for the Potomac River in the summer samples, we examined posterior probabilities of summer individuals assigned to the Shubenacadie River to determine whether overall confidence was lower than other groups. However, these posterior probabilities were comparably high. Over 90% of individuals were assigned to the Shubenacadie River with posterior probabilities higher than 0.80, and only 16 individuals fell below probabilities of 0.80. Thus assignment uncertainty in the Bayesian estimator could not account for the lack of Potomac River assignments.

By restricting our baseline to a subset of six source stocks, we calculated SDs around mixed stock estimates for the summer samples using the HISEA program in bootstrap mode. Mixed stock estimates (±1 SD) were 24.5% ± 3.5% for the Hudson River, 52.4% ± 4.8% for the Shubenacadie River, 19.8% ± 3.8% for the Potomac River, 2.4% ± 3.1% for the St. Lawrence River, and 0.4% ± 0.4% for the St. John River. These estimates are comparable to those identified by the ISMA program and suggest that variance around the MLE estimates was relatively low.

Discussion

The stock compositions of immature migrants in Minas Basin differed substantially from those reported for older

Fig. 3. Frequency distribution of maximum posterior classification probabilities for individual fish as determined by a Bayesian estimator (Munch and Clarke 2008). Results are shown for fish collected in (*a*) spring Maine trawls and (*b*) summer Minas Basin weir collections.



age classes. Dadswell et al. (1987) compiled data from several decades of tagging studies on adult American shad throughout the western Atlantic. Summer feeding aggregations in the upper Bay of Fundy contained individuals from stocks throughout their entire range, including Florida. Of adult migrants tagged in the Bay of Fundy, approximately 34% were recaptured in rivers south of Cape Hatteras (Region 1), 44% were recaptured between Cape Hatteras and Cape Cod (Region 2), 15% were recaptured in Bay of Fundy rivers (Region 3), and 7% were recaptured in the Gulf of St. Lawrence (Region 4). Dividing our composition estimates into similar regions and averaging across spring and summer collections, we estimated that the majority of 1-year-old migrants were from Region 2 (34%–47%) and Region 3 (52%–65%), with precise values depending on the statistical estimation method used. All collections contained less than 1% of fish from Regions 1 and 4, regardless of the estimator. Moreover, Regions 2 and 3 were each overwhelmingly represented by the Hudson River and the Shubenacadie River, respectively.

Several large northern populations of American shad were effectively absent from both the spring and summer samples. Fish from all extant Canadian stocks aside from the Shubenacadie River were either present in small numbers or were not detected. In addition, most rivers in the northeast United States contributed little to the samples. The lack of fish from these northern rivers was unexpected given their large historic population sizes. One possible explanation for their absences is the observation that many northern rivers, including the Connecticut, have experienced recent declines in year class strength (Atlantic States Marine Fisheries Commission (ASMFC) 2007). However, the Hudson River has experienced comparable drops in abundance indices to the Connecticut River (ASMFC 2007) and yet contributed a substantial number of fish to the assemblages here. Year class strength, therefore, does not appear to account entirely for different proportions of northern stocks in the mixtures. In addition, juvenile abundance indices are generally poorly correlated with subsequent abundances of adult spawners from the same cohort (ASMFC 2007), suggesting significant mortality and demographic restructuring during their time at sea (Limburg 2001). At least some of this restructuring may have already occurred prior to our collections, decoupling juvenile abundance from abundances of immature migrants.

Southern and midlatitude stocks were also generally absent from the mixtures, and most Chesapeake Bay stocks were not present in the collections. Stocks from south of Chesapeake Bay were also poorly represented in the mixtures, and no fish from Florida were detected. Unfortunately, juvenile abundances are not monitored in all rivers, and data are unavailable for most southern and Canadian rivers. Thus the influence of relative juvenile abundances for these rivers on our composition estimates is unknown. Inadequate sampling of the fish present in the Gulf of Maine and Bay of Fundy could bias the composition estimates if particular populations were present in the region but consistently missed by collections. However, we believe that this is unlikely given the temporal coverage of the weir collections where we collected samples continually between mid-June and early August. This corresponds to temporal abundance patterns in the region, with highest abundances typically occurring around early July (Dadswell et al. 1987). The Maine – New Hampshire trawl survey in 2005 occupied a total of 104 stations in a stratified random design covering some 13 000 km^2, and therefore we see no reason why these samples would be biased (Sherman et al. 2005). Finally, with the exception of the Potomac River fish, similar population compositions were detected in the Maine trawls and Minas Basin weir collections, despite alternative collection methods covering different dates and locations. Thus it is appears unlikely that the absence of many populations in our samples was due to sampling error.

The Potomac River was the only significant contributor from the Chesapeake Bay region, as identified by the MLE method for Minas Basin collections. While Potomac River stock abundance has been low for several decades, stocking efforts have enhanced the population, and juvenile abundance in 2004 was the largest recorded to date by monitoring surveys (ASMFC 2007). However, their presence in Minas Basin collections was equivocal given that the population was not identified in the sample by the Bayesian estimator. This discrepancy likely arose because of chemical similarities between baseline river-specific signatures, and the Bayesian estimator assigned these individuals to the Shubenacadie River instead of the Potomac River. Neither the Bayesian nor the MLE estimator showed evidence of high classification uncertainty, as demonstrated by the distribution of maximum posterior assignment probabilities for the Bayesian and small estimated variances for the MLE. Reduced classification confidence was therefore not the source of the discrepancy. Instead, these two estimators confidently assigned individuals with signatures falling on the boundary of two similar baselines to alternative sources. This result points to the need to interpret estimates of mixed stock composition with caution when baselines are similar.

The use of multiple independent estimators can help confirm the composition of mixed stocks and identify those sources that are equivocal.

Juvenile and immature American shad appear to exhibit high degrees of inter- and intra-population variability in the timing and extent of their migratory movements. Freshwater emigration timing can occur over a wide range of dates, with size and age playing a strong role in determining downriver movements (Limburg 1996). Further, both early and late emigrants can survive to spawn as returning adults (Limburg 2001), indicating juvenile migratory variability does not preclude successful future reproductive contributions. Movements may be similarly diverse after transitioning to coastal habitats. Juvenile emigrants have long been known to overwinter in nearshore estuarine environments (Hildebrand and Schroeder 1927; Milstein 1981; Hoffman et al. 2008). Others move more rapidly through estuaries to fully marine environments (Limburg 2001) and both subadults and adults have been captured across the shelf between 39°N and 41°N at depths of 50–100 m during winter months (Neves and Depres 1979). Finally, yearling American shad have been observed to make non-spawning movements into fresh water alongside spawning adults (Limburg 1998), further suggesting that some individuals remain in estuarine habitats and do not participate in coastal migrations during this immature life history stage. Our results support the hypothesis that American shad exhibit a diverse suite of migratory movements during the first year of life. Some individuals originated from nearby rivers (e.g., the Shubenacadie in Minas Bain collections), indicating minimal movement after freshwater emigration. Others derived from more distant sources (e.g., the Hudson), suggesting not all populations remain near their natal estuary during their first year. The current study focused on collections made in coastal habitats of the Gulf of Maine, and the identity of individuals collected from offshore environments remains to be determined. However, this work represents the first step in unraveling population-specific distributions of this highly migratory species during the immature life history stage.

Dadswell et al. (1987) described patterns of adult American shad migrations along the eastern coast of North America, whereby individuals from all populations throughout their range moved through the upper Bay of Fundy to capitalize on high local summer productivity and food availability. While Dadswell and co-workers observed this pattern for mature fish, our results showed a very different compositional pattern for immature migrants. Processes such as environmental variability, year class strength, and stock-specific behavior may collectively influence the composition of mixed-stock assemblages collected at any location and at any point in time. Interannual variability in assemblage composition was not addressed by this study, but temporal and ontogenetic changes in migratory behavior are certainly possible. Importantly, our data showed natal origin may play a role in determining the marine distributions of immature American shad. This variable behavior implies divergent exposure to suites of environmental stress, energetic costs, food availability, and predation pressure. The influence of population-specific marine distributions on latitudinal clines in life history traits in American shad is as yet unexplored.

There are major management implications of American shad movements and mixing in the marine environment. An assessment of mixed-stock compositions of coastal harvests off Maryland and Virginia based on mitochondrial DNA variation found significant variation in the contributions of individual stocks to the mixtures (Brown et al. 1999). This variation was both geographical and temporal, suggesting dynamic and unpredictable changes in the presence of specific stocks in harvests, with many stocks represented by numbers disproportionate to their population sizes. Such dynamism limits the ability to manage coastal mixed-stock fisheries without allowing unsustainable mortality of the most vulnerable stocks. Our work shows that the migratory habits of immature American shad are similarly complex, and managers of American shad should consider the potential impact of mixed-stock fisheries on early year classes. Immature fish are potentially more susceptible to harvest as bycatch in a variety of fisheries, imposing further anthropogenic stress on fully exploited stocks. Increased mortality of immature migrants in mixed-stock fisheries has the potential to substantially alter year class strength, restructure demographics, and further limit recovery of depleted populations. Combining results from studies employing traditional tags, morphometrics, DNA analysis, and now natural chemical tags in otoliths reveals the complex nature of American shad migrations in the marine environment.

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Fishing-induced evolution and changing reproductive ecology of fish: the evolution of steepness

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Abstract: Fishing can induce evolutionary changes in individual life history traits, leading to fish that mature smaller and younger and with larger gonads, so that they reproduce more intensely. The steepness of a stock–recruitment relationship is commonly defined as the fraction of recruitment of an unfished population obtained when the spawning stock biomass is 20% of its unfished level. We use a model of harvest-induced evolutionary change to understand how the steepness of the stock–recruitment relationship changes due to fishing. If the true spawning stock biomass is known, the stock–recruitment relationship changes little under fishing-induced evolution and there is little concern for fisheries management. When management is based on a total biomass – recruitment relationship, recruitment may be underestimated, which is also of little concern from a sustainability perspective. However, when the number of spawners – recruitment relationship is used to forecast recruitment, management practice that ignores the evolution of steepness may overestimate recruitment and therefore recommend catches that exceed safe biological limits. Using outdated maturity ogives underestimates spawning stock biomass, which results in steeper and higher stock–recruitment relationships as life histories evolve. Although of little concern for sustainability, this may pose challenges for practical fisheries management.

Résumé : La pêche peut provoquer des changements évolutifs dans les traits individuels du cycle biologique et entraîner l'existence de poissons qui atteignent la maturité à un âge plus précoce et une taille réduite, mais avec des gonades plus grandes, si bien que leur reproduction est plus intense. Le degré d'inclinaison de la relation stock–recrutement est couramment définie comme la fraction du recrutement d'une population non exploitée obtenue lorsque la biomasse du stock reproducteur est à 20 % de son niveau non exploité. Nous utilisons un modèle de changement évolutif généré par la récolte pour comprendre comment le degré d'inclinaison de la relation stock–recrutement change à cause de la pêche. Si la véritable biomasse du stock reproducteur est connue, la relation stock–recrutement change peu avec l'évolution causée par la pêche et il y a peu de préoccupations à y avoir pour la gestion de la pêche. Dans les cas où la gestion est basée sur la relation entre la biomasse totale et le recrutement, le recrutement peut être sous-estimé, ce qui est aussi peu préoccupant dans une perspective de durabilité. Cependant, lorsqu'on utilise la relation entre le nombre de reproducteurs et le recrutement pour prédire le recrutement, une méthode de gestion qui ne tient pas compte l'évolution du degré d'inclinaison peut surestimer le recrutement et ainsi recommander des captures qui dépassent les limites biologiques sûres. L'utilisation d'ogives de maturité périmées sous-estime la biomasse du stock reproducteur, ce qui produit des relations stock–recrutement plus abruptes et plus hautes à mesure que les cycles biologiques évoluent. Bien que ce soit peu préoccupant pour la durabilité, la situation peut poser des défis pour la gestion pratique de la pêche.

[Traduit par la Rédaction]

Introduction

There is increasing recognition that fishing can be a source of artificial selection and lead to evolutionary change in a variety of traits (to our knowledge, first suggested by Rutter (1902) and then revived by Law and Grey (1989), and the recent focus is exemplified by Jørgensen et al. (2007) and Dunlop et al. (2009*a*)). In particular, life history characteristics have been suggested to be especially prone to change (Ernande et al. 2004; Heino and Dieckmann 2008). Although there is still a dispute about the magnitude of such effects (Brown et al. 2008; Hilborn and Minte-Vera 2008; Andersen and Brander 2009), a precautionary approach requires that we consider the effect of fishing-induced evolu-

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tion when planning human intervention into marine ecosystems (Jørgensen et al. 2007); such an approach has been called "evolutionarily enlightened management" (Ashley et al. 2003).

Since Darwin (1859), we have understood the strong effects that artificial selection can have, and there is every reason to believe that fisheries as a source of artificial selection will act on reproductive biology (Rijnsdorp 1993). Integrating reproductive biology into scientific advice for fisheries management continues to be a challenge (Trippel 1999; Morgan 2008; Mangel et al. 2010), and in this paper, we address one important concern by analyzing how fishing-induced evolution will act on the reproductive biology of targeted species.

In this paper, we investigate how the relationship between the biomass of reproductively mature individuals (spawning stock, S) and the resulting offspring added to the population (recruitment, $R(S)$) responds to the selective pressure induced by fishing. When recruitment approaches an asymptote when stock size is large, the Beverton–Holt stock–recruitment relationship (Beverton and Holt 1957–1993)

$$(1) \qquad R(S) = \frac{\alpha S}{1 + \beta S}$$

is commonly used. In eq. 1, α is the maximum per capita productivity and β is a measure of intensity of density dependence. The functional relationship in eq. 1 can be derived by assuming that the per capita mortality rate of the recruited class is a linear function of their numbers (Beverton and Holt 1957–1993; Mangel 2006). Here, we derive the stock–recruitment relationship from more fundamental biological mechanisms using a previously published individual-based model for Atlantic cod (*Gadus morhua*) (Enberg et al. 2009). This approach allows us to study fishing-induced evolution of the stock–recruitment relationship. That is, we seek to predict the stock–recruitment relationship based on the evolutionary ecology and associated life history parameters of a species and thus understand how steepness (and the stock–recruitment relationship itself) will change in response to harvesting.

Predicting stock–recruitment relationships from evolutionary ecology is of more than academic interest. For example, strategic fisheries management requires estimates of biological reference points that often critically depend upon the nature of the stock–recruitment relationship (Brodziak 2002). The Beverton–Holt stock–recruitment relationship, in which recruitment reaches an asymptote due to increased density dependence, is widely used in stock assessments of marine fishery resources. Furthermore, the recovery of threatened or endangered populations is in large part determined by the resilience of reproduction, which in turn depends on the stock–recruitment relationship.

We use a model for Atlantic cod to illustrate our ideas because cod are commercially valuable, highly productive, and intensively exploited fishery resources and there is evidence of fishing-induced evolution in several stocks (Northeast Arctic cod (Heino et al. 2002*b*), North Sea cod (Yoneda and Wright 2004), and Canadian cod stocks (Olsen et al. 2005; Swain et al. 2007)). In the next section, we describe our model (first described in Enberg et al. 2009) and how it produces the relationship between the biomass of the population or the number of spawning individuals and the number of recruits and how these change under fishing-induced evolution. Here we use the commonly accepted definition (Mace and Doonan 1988) that steepness (h) is the recruitment (R) at 20% of unfished biomass relative to recruitment at unfished biomass (S_0), that is,

$$(2) \qquad h = \frac{R(0.2\,S_0)}{R(S_0)}$$

We explore how steepness may evolve with harvesting and explain why it changes the way it does. Several stock–recruitment relationships are used in fisheries management, often dictated by the availability of data, and we therefore show changes in steepness for alternative formulations in which S may represent spawning stock biomass, total biomass, or the number of spawners. We then describe our main result: that the stock–recruitment relationship changes due to fishing-induced evolution, but that the direction and magnitude of change depends on which proxy is used for the stock indicator S. Via a sensitivity analysis, we show that our result is robust and not caused by a special parameter combination. We then discuss possible consequences of ignoring changes in the stock–recruitment relationship, with practical relevance for fisheries management.

Materials and methods

In our analysis, we use an evolving, individual-based model parameterized for a long-lived and late-maturing fish species such as Atlantic cod in its northern range. The model has already been presented in detail in Enberg et al. (2009). Here we give an overview over the main processes, but to improve readability, we have omitted equations as far as possible. The main focus is on individual bioenergetics and evolution of life history traits with consequences for population dynamics, and the model includes the processes of (*i*) growth, (*ii*) sexual maturation, according to a linear probabilistic maturation reaction norm (PMRN) (Heino et al. 2002*a*), and (*iii*) mating and offspring production. For growth, a model is used that includes acquisition of food and allocation to somatic and gonadic growth (Quince et al. 2008; see also Enberg et al. 2009). An allometric scaling exponent of 0.75 is used, which is suitable for piscivores. In addition, food acquisition is density-dependent (lower if the population is large, reflecting increased competition for food) and variable over time (temporally autocorrelated) and between individuals. For sexual maturation, a PMRN describes the probability that an individual of a given size and age becomes sexually mature and thus incorporates the environmental influence on maturation through phenotypic plasticity. This method is frequently used to quantify changes in maturation tendencies in wild fish populations (reviewed by, e.g., Dieckmann and Heino 2007) and in modeling studies of fishing-induced evolutionary change (Dunlop et al. 2009*b*). In mating and offspring production, the number of offspring that an individual produces and its probability of becoming a mate are proportional to its gonad size. Beyond this, there is no mate choice, so sexual selection is excluded. The offspring inherits the midparental trait

value with some noise added around it to give heritabilities of maturation age and size around $h^2 = 0.2$ (Gjedrem 1983).

Each individual has four life history traits inherited from its parents that therefore may evolve with time: (1) intercept and (2) slope of the linear PMRN; (3) a growth coefficient that determines growth rate in a trade-off with natural mortality and thus represents risk-sensitive foraging behavior; and (4) the target gonadosomatic index (GSI; ratio of gonads to total body mass), which determines the intensity of reproductive investment. A higher GSI implies that more of the total resources are allocated to reproduction and less to somatic growth.

The modeled individuals determine and are regulated by population-level feedback processes, including Beverton–Holt density-dependent recruitment and density-dependent growth resulting from resource competition. In addition, the feeding environment fluctuates in time to represent cold vs. warm or productive vs. unproductive periods. Finally, each individual has a risk of dying that depends on natural mortality (declining with size) and fishing mortality (sigmoid selectivity curve as is typical for trawls, with 50% selectivity at 85 cm body length).

We provide a diagram that illustrates how the processes in the model relate to one another (Fig. 1). A central assumption is that a Beverton–Holt function describes the relationship between the number of eggs spawned and the number of recruits surviving to age 1. In this paper, we report the effects on stock reproduction and steepness, as these population-level characteristics originate from evolving individual strategies, and their effects on population structure and density-dependent feedback. This analysis has both an ecological dimension, as the feedback processes depend on the number, states, and traits of individuals in the population, as well as the environmental variability, and an evolutionary dimension because selection pressures may cause the individual traits to change from generation to generation.

Thus, a key to analyzing evolutionary effects on steepness is the separation of ecological and evolutionary processes. In particular, as an evolving population is fished for increasing durations of time, individual traits gradually adapt to the fishing pressure. At different points in time, we freeze the evolutionary process and study the ecological relationships in a population with those traits. That we isolate ecological dynamics in this way does not imply that the environment needs to be constant; in our case, it is fluctuating and we focus on the average response over time. This also means that in this model, fishing is the sole driver of long-term change. Because steepness also depends on population structure, we derive the stock–recruitment relationship for two typical ecological scenarios: a fish stock currently fished (dominated by young and small individuals) or a fish stock with a moratorium implemented so that more larger and older fish are in the population.

The analysis on how fishing-induced evolution affects the steepness of the stock–recruitment relationship is carried out in the following steps (see also the flow chart in Fig. 2). First, we initialized a population with random traits and let it adapt to natural mortality alone for 200 000 years. This ensures that the population approaches an evolutionary equilibrium analogous to that of the cod stock prior to any harvesting. We then let this population adapt for a further 5000 years to natural mortality alone, and this was done 20 times so that we have 20 replicates, each starting from different initial conditions.

In step 2, the 20 replicate populations were harvested at a fixed rate of 0.4·year^{-1} using a sigmoidal size selectivity curve resembling a trawl fishery. We assumed that selectivity increases rapidly for fish with a body size of 85 cm, which corresponds to the mean body length at maturation before the start of harvesting. We stored the distribution of evolving traits after (*i*) 0, (*ii*) 50, (*iii*) 100, (*iv*) 200, and (*v*) 400 years. In this way, we built a library of 20 replicate populations of each harvest duration, 100 populations in total. By averaging over the 20 replicates, our results represent the expected mean evolutionary outcome (we observed no bimodalities in trait distributions or evolutionary outcomes).

In step 3, we then ran each population (*i*–*v*) without evolution for 150 years, so that offspring traits were not inherited from parents but drawn from the library from step 2. This was accomplished in the recruitment process by replacing the predicted recruits at each time step with a fixed number of seeded recruits, with each offspring's life history traits drawn randomly from the population assembled in step 2. We repeated this process 20 times for each of the population libraries above and with a fixed number of seeded recruits in nine logarithmically spaced steps up to 60 000 recruits annually (Fig. 3). The purpose of different recruitment levels was to sample the stock–recruitment curve under different population densities, and the 20 replicates were to average over ecological variability. We did so both in the absence of harvesting and with harvesting, because the stock–recruitment relationship may differ as fishing changes the population structure.

In step 4, of the 150 years simulated in step 3, we allowed 100 years to reach a fluctuating equilibrium for the population's abundance and trait distribution before we stored results and averaged them over the last 50 years of simulation. Thus, each stock–recruitment curve that we present is based on nine points of increasing population size, with each point being an average of the last 50 years in $20 \times 20 = 400$ simulations. We stored different properties of the stock (number of spawners, spawning stock biomass, and total biomass), as well as the number of recruits predicted from the modeled processes of growth, survival, and reproduction. These stored data are used to present alternative formulations of the stock–recruitment relationship.

In step 5, the steepness of each stock–recruitment curve was calculated using eq. 2 so that $h_x = R_x(0.2S_0)/R_0(S_0)$, where the subscript x on h and R indicates that we used the populations that had been adapted to harvesting for x years, and S_0 indicates that the spawning stock size is at its average level for the preharvest situation. Note that this formulation assumes that the average stock size S_0 and the corresponding average recruitment level $R_0(S_0)$ for the pristine stock are known and that steepness is defined as recruitment in the current population at a stock size of $0.2S_0$. In practice, $R_x(0.2S_0)$ was computed from the fishing-affected stock–recruitment curve using curvilinear Lagrange interpolation, and this was repeated for each duration of harvesting, i.e., $R_0(0.2S_0)$, $R_{50}(0.2S_0)$, $R_{100}(0.2S_0)$, $R_{200}(0.2S_0)$, and $R_{400}(0.2S_0)$.

In step 6, we also calculated maturity ogives (the proportion of the stock that is sexually mature as a function of age

Fig. 1. The modeling cycle is divided into individual-level and population-level processes. From the top right, in each year for all immature individuals, current age and size determine the probability of becoming mature according to a linear probabilistic maturation reaction norm (PMRN). The first two evolving individual traits (these are marked in the flowchart with numbers) are (1) the intercept and (2) slope of the PMRN. When mature, growth changes because resources also have to cover gonad maturation. Growth is also density-dependent, it fluctuates over time, and individuals experience slightly different growth conditions, and an evolving trait determines (3) the growth coefficient in a trade-off in which faster growth leads to higher mortality. Natural mortality declines with body length, whereas fishing mortality is modeled with sigmoid size selectivity as for trawling. An evolving trait also describes (4) reproductive investment implemented as the target gonadosomatic index. Offspring are produced according to gonad size, with offspring traits inherited from the parents. When these processes have been repeated for each individual, the population characteristics are updated. The total egg production determines the density-dependent juvenile survival. Recruitment feeds into population dynamics over time. The total biomass of the population determines density-dependent competition for food, which feeds back to the growth rate. By repeating this for several years, the four life history traits adapt to the fishing regime, and individual and population characteristics can be quantified and analyzed. For further model details, see the text and Enberg et al. (2009).

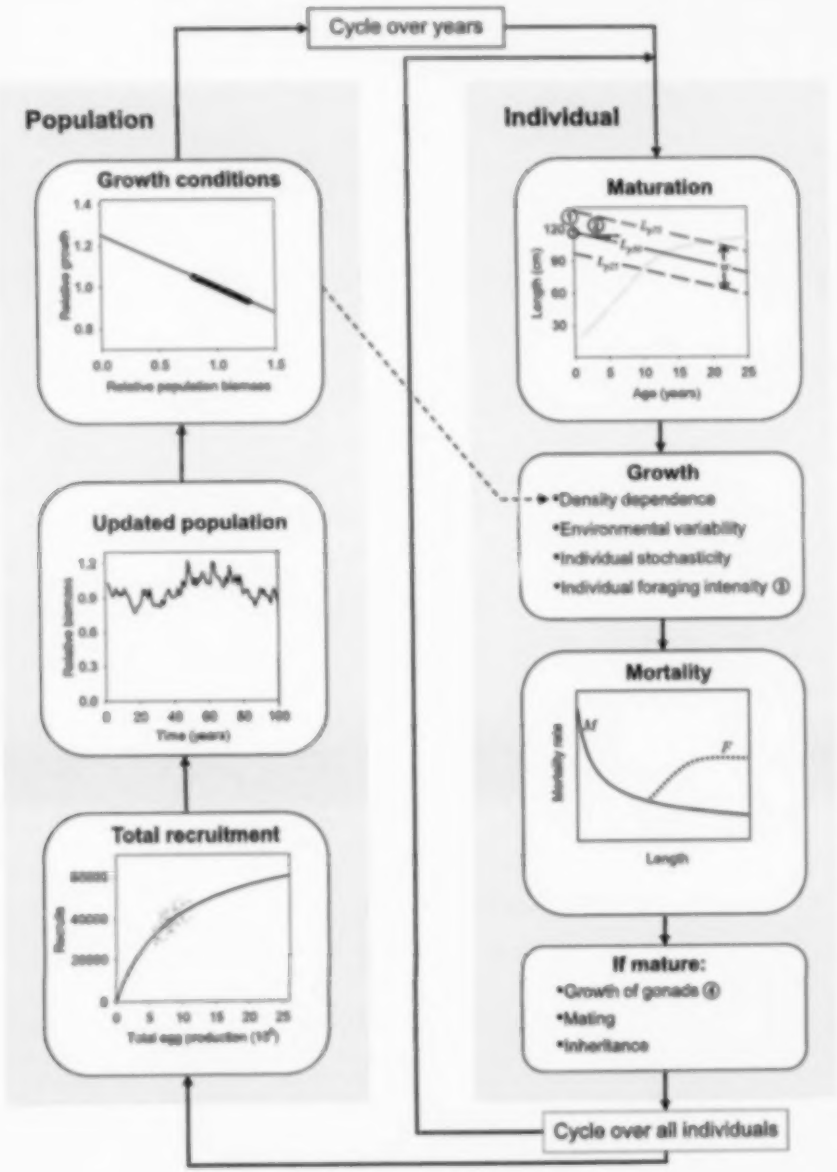
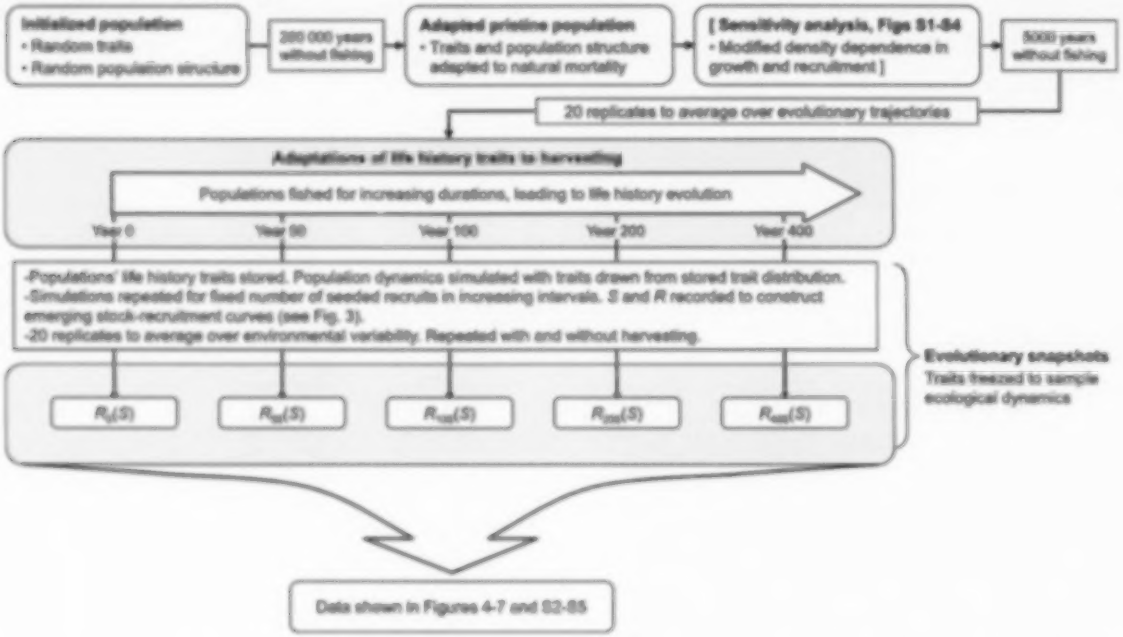


Fig. 2. Flow scheme showing how model simulations were performed to quantify changes in the emergent stock–recruitment curves. See Supplementary data for Figs. S1–S5.[4]


Initialized population
• Random traits
• Random population structure
200 000 years without fishing
Adapted pristine population
• Traits and population structure adapted to natural mortality
[Sensitivity analysis, Figs S1-S4
• Modified density dependence in growth and recruitment]
5000 years without fishing
20 replicates to average over evolutionary trajectories
Adaptations of life history traits to harvesting
Populations fished for increasing durations, leading to life history evolution
Year 0
Year 50
Year 100
Year 200
Year 400
-Populations' life history traits stored. Population dynamics simulated with traits drawn from stored trait distribution.
-Simulations repeated for fixed number of seeded recruits in increasing intervals. S and R recorded to construct emerging stock-recruitment curves (see Fig. 3).
-20 replicates to average over environmental variability. Repeated with and without harvesting.
Evolutionary snapshots
Traits freezed to sample ecological dynamics
$R_0(S)$
$R_{50}(S)$
$R_{100}(S)$
$R_{200}(S)$
$R_{400}(S)$
Data shown in Figures 4-7 and S2-S5

or length) for the populations to study common measures of maturation used in fisheries: age at 50% maturity (A_{50}) and length at 50% maturity (L_{50}). We found maturity ogives using a general linear model with a logit link function on proportions mature as a function of age, $o(a) = [1 + \exp[-\beta(a - A_{50})]]^{-1}$ by fitting parameters β and A_{50}, and correspondingly for size, $o(l)$.

Finally, in step 7, we tested a common source of error in practical fisheries management, i.e., using outdated maturity ogives to calculate SSB from survey data on population size or age distribution. To do this, we used the preharvest maturity ogives for calculating spawning stock biomass based on numbers at age (in years) or length (in bins of 2 cm) combined with average weight at age or length. Thus, if $n(a)$ denotes the number of fish at age a, $o(a)$ denotes the proportion mature at age a, and $w(a)$ denotes the mean weight of fish at age a, then

$$(3) \qquad \mathrm{SSB} = \sum_a n(a) \cdot o(a) \cdot w(a)$$

The rationale here is that $n(a)$ and $w(a)$ are often routinely monitored in research surveys, whereas $o(a)$ often requires more thorough analyses of gonad stage and is updated less frequently. By using old data (from the pristine stock) for maturity, i.e., $o_0(a)$ but updated observations as the stock evolves, for example, $n_{100}(a)$ and $w_{100}(a)$, we can thus quantify the consequences of using old maturity data. We used an analogous formula for data stratified by length rather than by age.

The sensitivity of our results to the assumptions that we have made for the strength of density dependence in growth and recruitment are given in Supplemental Figs. S1–S4.[4] We also evaluate consequences of using outdated maturity ogives when estimating SSB from surveyed age or length distributions (Supplemental Fig. S5)[4].

Results

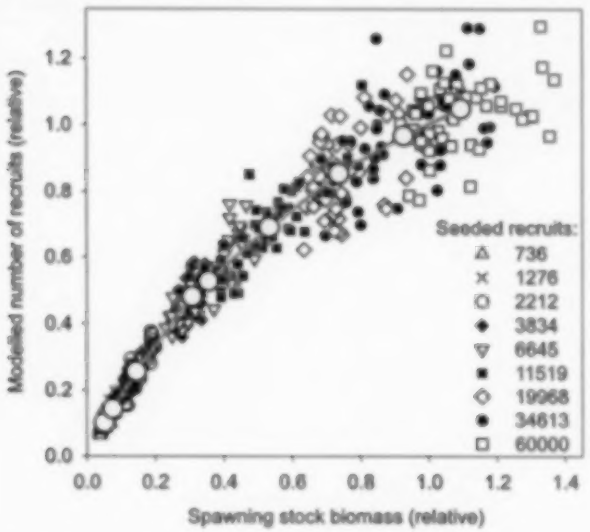
Evolution of stock–recruitment relationships

Fishing-induced evolution has consequences for the stock–recruitment relationship, but the direction and magnitude of the change depends on the type of stock–recruitment relationship used (Fig. 4). The underlying cause that explains these systemic changes relates to evolution of the population's life history traits. The most dominant change in this model was that fishing induced evolution toward earlier maturation, which increased the proportion of the total biomass that is mature. If total stock biomass is used as proxy, a larger proportion of it will reproduce, and recruitment will therefore become higher with time. This explains the increasing steepness that our model predicts for the total stock biomass – recruitment relationship (Fig. 4*a*). Here, fishing-induced evolution lifts the entire stock–recruitment relationship over time, and the slope close to the origin becomes steeper (Figs. 4*a*, 4*d*).

In contrast, when spawning stock biomass is used as proxy, the stock–recruitment relationship remains almost unchanged (Figs. 4*b*, 4*e*). This is because the efficiency with which mature fish produced gonads and fertilized eggs varies much less. A tonne of spawning stock biomass produces roughly the same biomass of gonads and approximately the same number of fertilized eggs, even when life history

[4] Supplementary data for this article are available on the journal Web site (http://cjfas.nrc.ca).

Fig. 3. Example of how each stock–recruitment curve was produced by using different fixed numbers of seeded recruits to sample different regions of the curve, corresponding to different population densities. During population simulations, the stock proxy (here spawning stock biomass) and the model's predicted number of recruits were recorded and are plotted here. Evolutionary trait inheritance was turned off when these curves were produced so that each offspring received the traits of a random individual from a stored population that represents a frozen point in time. The symbols represent different fixed numbers of seeded recruits (see inset legend), and the line is drawn between the average value (shaded circles) of each recruitment level. In Figs. 4 and 7 and in Supplemental Figs. S2–S4,[4] only the average lines are shown. The example here is recruitment of the pristine population, $R_0(S)$, without harvesting.



evolution results in SSB consisting of smaller individuals. Therefore, if one can keep track of the part of the total biomass that is sexually mature, then that proxy will remain a pretty accurate predictor of recruitment, despite large changes in life history traits. So, although the composition of the spawning stock biomass is changing toward more numerous and smaller-bodied fish in the model, their weight-specific reproduction remains largely unchanged (Fig. 4*b*). It should be noted that the relationship shown here assumes that the correct spawning stock biomass is known, which requires perfect knowledge about which fish are mature; we relax this assumption below.

For some stocks such as anadromous salmonids, the spawners are counted as they return to spawn. This proxy ignores that as fish mature at smaller body size with time, they also have smaller gonads. A given number of fish will therefore produce less biomass of gonads as they adapt to fishing and, consequently, fewer fertilized eggs. This is why the number of spawners – recruitment relationship is shifted downwards with time, and the slope close to the origin becomes less steep (Figs. 4*c*, 4*f*).

Moratorium versus continued fishing

Continued harvesting on a stock has two implications. First, the repeated fishing events will take away the older and larger individuals so that the stock is biased towards young and small fish. Second, fishing releases density dependence by removing biomass so that individuals grow faster, and when fewer spawners produce fewer eggs, the Beverton–Holt juvenile density dependence prescribes that each egg has higher survival probability. Together, released density dependence during fishing may increase the population's ability to grow. When we contrast results as they would have been observed if fishing were continued or a moratorium put in place, it is the combined role of demography and density dependence that is quantified.

The spawning stock biomass – recruitment relationship is only negligibly affected by fishing-induced life history evolution over time, but the whole relationship is less steep if observed during a moratorium (compare Figs. 4*b* and 4*e*). This is because biomass builds up during the moratorium, leading to reduced individual growth and reduced survival from egg to recruit. As a consequence, the same recruitment level requires a higher spawning stock biomass in the moratorium scenario compared with when fishing is continued.

The total biomass – recruitment relationship is less influenced by life history evolution during the moratorium scenario than during the fished scenarios (Figs. 4*a*, 4*d*). Prior to life history evolution, the size-selective fishing takes out mostly mature fish, and fishing therefore has a large effect on reproduction (the solid lines are very different between Figs. 4*a* and 4*d*). When the population has adapted to fishing, the fish mature smaller and spend more of their life below harvested sizes. In that case, continued fishing has less impact on the mature proportion, and the total stock biomass – recruitment relationship is more similar between the moratorium scenario and fished scenarios (compare dotted lines in Figs. 4*a* and 4*d*).

With number of spawners as proxy, evolution leads to stock–recruitment curves that are less steep close to the origin for both the moratorium and the fished scenarios (Figs. 4*c*, 4*f*). Populations adapted to harvesting mature at smaller size and therefore stay below the harvested sizes for more of their life. A given number of spawners will therefore be of smaller size, have smaller gonads, and produce fewer recruits as evolution proceeds; this is why the number of spawners – recruitment curves become less steep.

Changes in steepness parameter *h*

The changes in the stock–recruitment relationships can be quantified by the steepness parameter h (eq. 2; Fig. 5). Steepness changes the least when actual spawning biomass is known, becomes steeper when the stock is expressed in terms of total biomass, and becomes less steep if the number of spawners is used. Regardless of whether fishing is continued or stopped, the change in steepness is roughly the same if spawning stock biomass or number of spawners are used as proxies in the stock–recruitment relationship. When total stock biomass is used, however, the increase in steepness is more pronounced if fishing continues (compare Figs. 5*a* and 5*b*).

The longer the population is subject to harvest, the more life history evolution will take place, and thus the greater the change in steepness will be. Moreover, the changes in steepness are most rapid early on, and for all three stock–recruitment relationships, the value of steepness changes

Fig. 4. The consequences of fishing-induced evolution for stock–recruitment relationships depend on the stock property that is surveyed and used to monitor and predict recruitment (indicated on the *x* axes). These panels show recruitment as a function of (*a* and *d*) total stock biomass (age 2+), (*b* and *e*) spawning stock biomass, and (*c* and *f*) number of spawners. In the upper panels, the stock is being fished while the sampling is carried out, whereas in the lower panels, there is no fishing during sampling, corresponding to observations that might be made during a fishing moratorium. All values are normalized relative to the mean equilibrium value of the proxy and recruitment prior to life history evolution and in the moratorium situation (pristine stock dynamics).

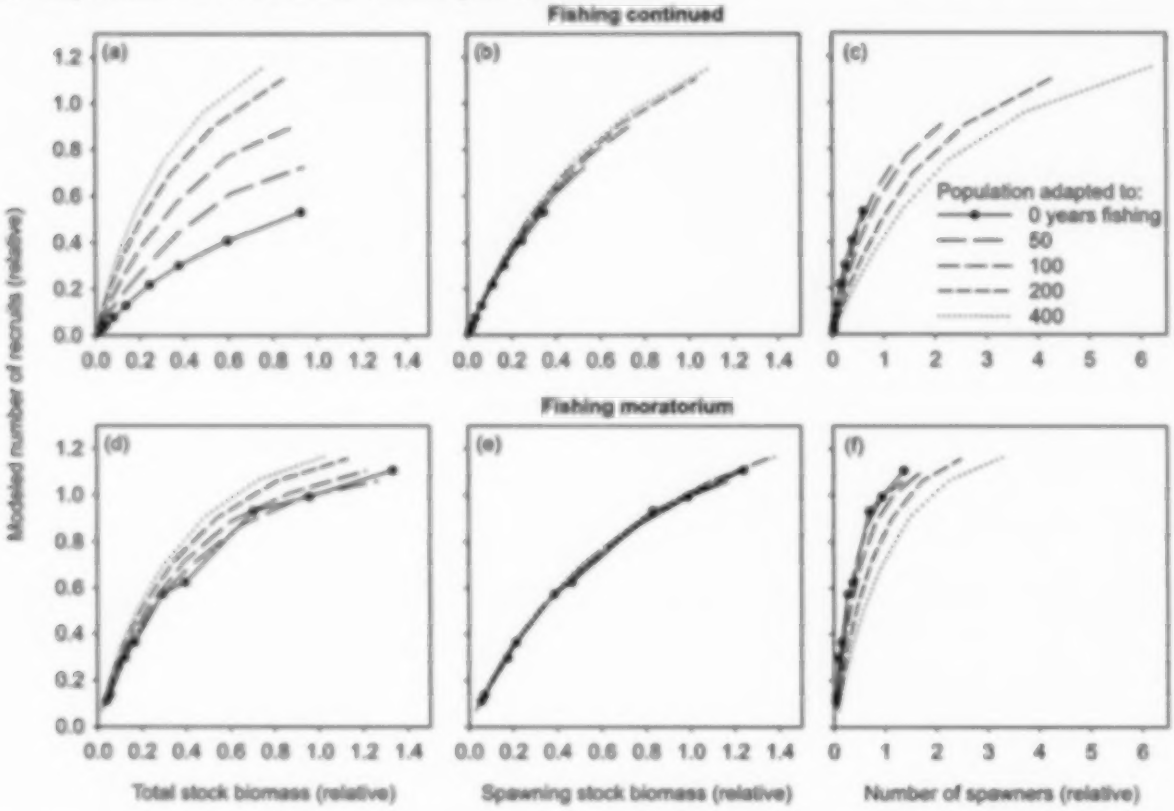


Fig. 5. Fishing-induced evolution of the steepness parameter *h*, quantified for different stock–recruitment relationships, as observed (*a*) if fishing continues on the stock or (*b*) if a fishing moratorium is implemented. The model population was harvested for increasing duration (*x* axis), and population dynamics were analyzed to quantify the stock–recruitment curve and the steepness parameter. The different lines correspond to stock–recruitment relationships that use different proxies for parental stock (triangles and dotted lines, total stock biomass; circles and solid lines, spawning stock biomass; squares and broken lines, number of spawners).

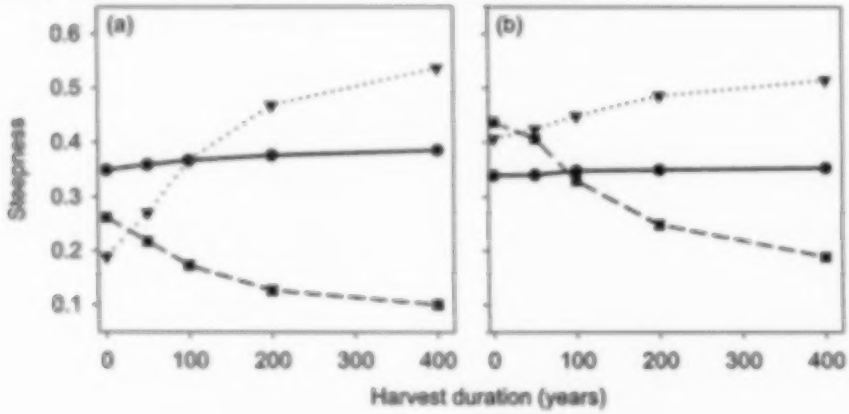
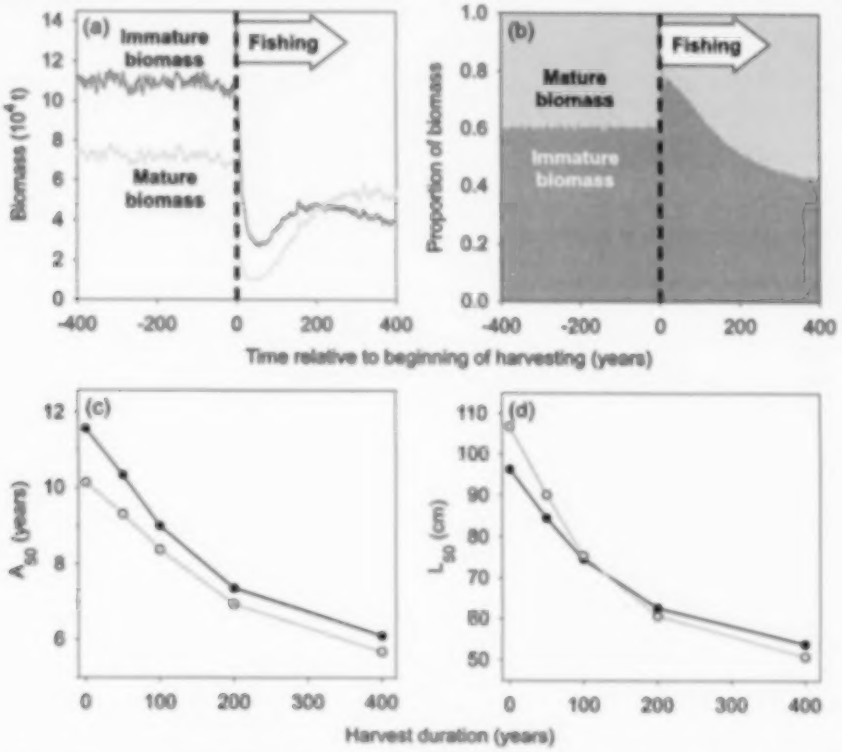


Fig. 6. (*a*) The development of mature (spawning stock) biomass and immature biomass due to demographic changes and evolution of life history traits; (*b*) the same presented as proportion of total biomass. In (*a*) and (*b*), there is no fishing prior to year 0, and the preharvest equilibrium is shown for 400 years to illustrate the level of interannual variability in the model. The biomass changes are caused by maturation evolution, as evidenced by declining (*c*) age at 50% maturity (A_{50}) and (*d*) length at 50% maturity (L_{50}) as a function of the duration of harvesting. Shaded dots show values that would be seen if fishing were continued, whereas solid dots show the values that would be observed during a moratorium.



more slowly with time. However, new equilibria are not reached even after 400 years of harvesting.

Life history evolution and changing steepness

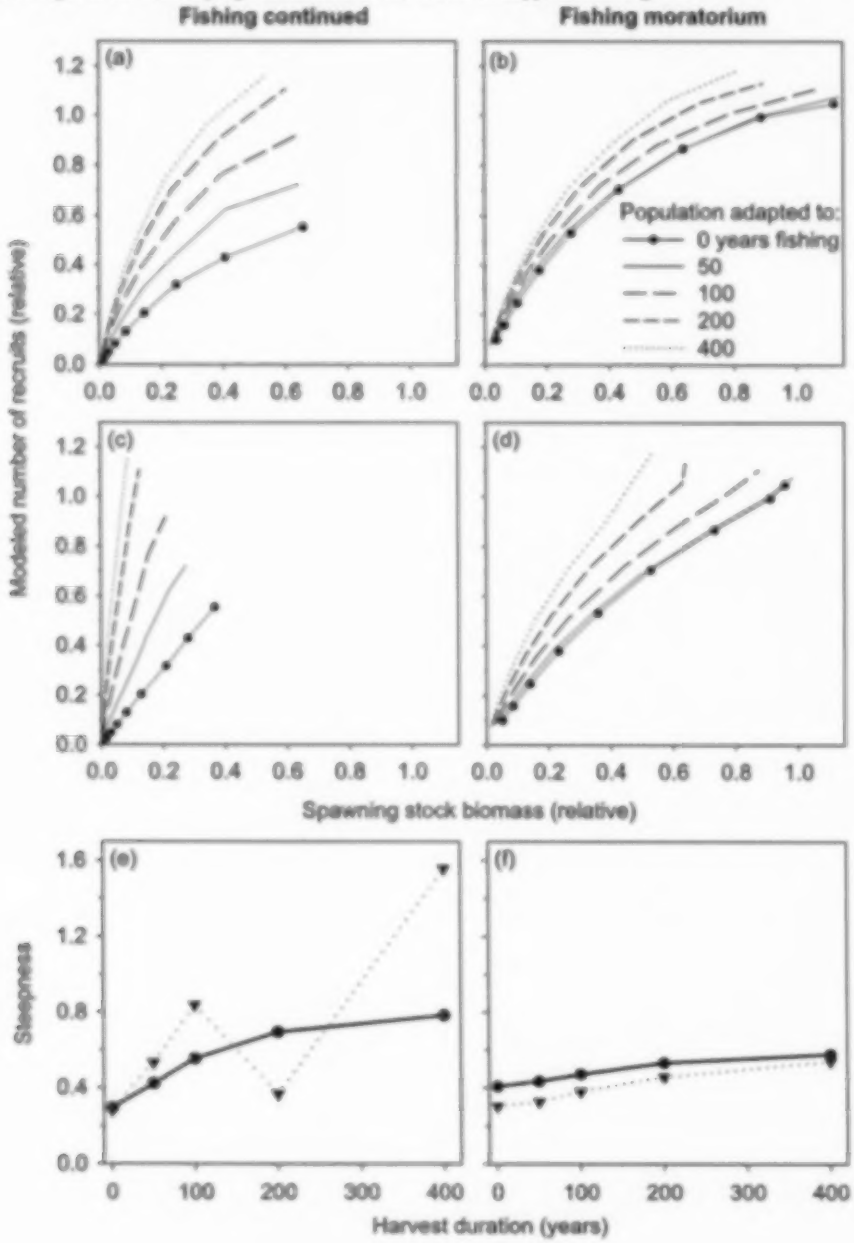
The evolutionary changes in steepness and the stock–recruitment relationships can be understood by investigating population dynamics and individual life history traits. When harvesting begins on a stock, population size declines, especially the number of old and sexually mature fish (Fig. 6*a*). After some period of harvesting, however, total stock biomass and mature biomass begin to increase (Fig. 6*a*). This happens because the life history traits evolve adaptations to the new fishing regime. In particular, earlier maturation allows individuals to mature before they are fished, and fish that mature smaller also spend more time below the minimum size limit in the fishery. The proportion of the stock that is comprised of mature fish increases over time, from 40% in the preharvest equilibrium to around 60% after a few centuries of harvesting (Fig. 6*b*). The underlying evolutionary changes in life history traits in the model cause age and length at maturation to decline (Figs. 6*c*, 6*d*), and the fish also have larger gonads for a given size and grow faster. Compared with the situation before harvesting, these evolutionary changes in individual life history traits lead to fish that mature smaller and younger and with larger gonads, so that they reproduce more intensely.

When maturation is surveyed infrequently

Thus far, we have used the model output directly, which means that we assume perfect knowledge about the stock. One particular source of error is when old maturity ogives are used to estimate spawning stock biomass. To mimic this situation, we used a maturity ogive from the pristine stock (before harvesting) to calculate spawning stock biomass from age or length distributions (using eq. 3), although evolution of the stock's life history traits would cause changes in the maturity ogives. The spawning stock – recruitment curves estimated in this way become steeper over time, regardless of whether age or length ogives are used and regardless of whether fishing is continued or a moratorium implemented (Figs. 7*a*–7*d*). This result shows stark contrast to the situation in which accurate information about stock maturity is known; there the spawning stock – recruitment relationship is essentially constant over time (Figs. 4*b*, 4*e*).

A note of caution should be added: the length-based maturity ogives are statistically fitted logistic curves assuming a sigmoid shape, but this functional form does not fit very well with the actual proportions mature as a function of length (for examples, see Supplemental Figs. S5*b* and

Fig. 7. When the spawning stock biomass is calculated using a maturity ogive derived from the pristine stock, the SSB–recruitment curve becomes steeper as evolution progresses with the duration of harvesting. The SSB has been calculated based on an age-based maturity ogive in (*a*) and (*b*) and on a length-based maturity ogive in (*c*) and (*d*). (*e*) and (*f*) The consequences for steepness are shown, with circles and solid lines representing steepness when an outdated age-based maturity ogive is used, and triangles and dotted lines representing length-based ogives. In (*a*), (*c*), and (*e*), observations are made on a stock that is continuously fished; in (*b*), (*d*), and (*f*), there is a fishing moratorium. The temporal changes in the maturity ogives themselves are shown in Supplemental Fig. S5.[4]



S5*d*[4]). The poor fit results in spawning stock – recruitment curves that are unrealistically high when length-based ogives are used and makes estimation of steepness difficult and potentially inaccurate (Figs. 7*e*, 7*f*). The same complications do not apply to the age-based maturity ogives in our model.

Discussion

Human interventions may change the environment to which organisms adapt in ways that trigger evolutionary processes on multiple time scales (Reznick and Ghalambor

2001). These adaptations may be quick and take place within decades (Hendry and Kinnison 1999; Reznick and Ghalambor 2001). For fish, one element of their environment that has changed dramatically in recent times is that the harvest rates imposed by commercial fishing may be up to four times higher than natural mortality rates (Mertz and Myers 1998). This led Rijnsdorp (1993, p. 391) to call fisheries "a large-scale experiment on life-history evolution". A recent meta-analysis identified harvesting as producing rates of phenotypic change roughly three times higher than other anthropogenic interventions (Darimont et al. 2009), and another meta-analysis found that life history changes were of larger magnitude in fish populations subject to more intense harvesting (Sharpe and Hendry 2009). As time series data and tools to analyze them have become available, life history traits and, particularly, age and size at maturation have been identified as particularly prone to change (Heino and Dieckmann 2008). These traits are central to fish population dynamics as they determine when fish become reproductively active and, therefore, how efficient the population is at producing new recruits. Such fishing-induced life history evolution may therefore be crucial for medium- and long-term development of yield and for fisheries management (Jørgensen et al. 2007).

Stock–recruitment relationships

In most cases of practical fisheries management, it is impossible to quantify individual reproduction directly. Instead, over many years, one records the relationship between population-level characteristics and recruitment to the stock, and management then aims to keep the population-level measure within limits that ensure acceptable recruitment (Fowler 2009). Examples of such population-level characteristics used in fisheries management include total stock biomass, spawning stock biomass, and number of spawning fish. Thus, although the aim of fisheries management is to ensure sustainable levels of recruitment, this is often obtained by focusing management on a proxy that is easier to quantify during surveys. An additional advantage is that there is often a time lag between the population-level measure and recruitment, so this approach allows for forecasts for the near future.

The stock–recruitment approach rests on the assumption that the past relationship between the population-level proxy and recruitment remains unchanged, so that the stock's past behavior describes what it will do now and in the near future. Because fishing-induced evolution has potentially potent effects on individual life history traits, that assumption may be violated.

Consequences of changing steepness

In this paper, we used an individual-based model for fish life history evolution to study the evolution of reproductive characteristics as they respond to harvesting. In particular, we quantified changes in the stock–recruitment relationship and the steepness parameter that is often used in fisheries management.

If the stock–recruitment relationship becomes steeper with time, then recruitment will be higher than predicted from the population-level proxy. From a sustainability perspective, this is of little concern because recruitment will be better than believed by the managers. In practical fisheries management, this may still cause conflicts, because fishermen will observe the stock as repeatedly being larger and recruitment higher than that forecasted by the managers. Stakeholders in the industry may then call for higher quotas, which managers may feel uneasy about because they believe that recruitment should be worse than it is. Our model suggests that this will be the case if recruitment is modeled with a total biomass – recruitment relationship or if older length- or age-based maturity ogives are used to estimate the spawning stock biomass.

The situation is more serious if the stock–recruitment relationship becomes less steep over time. Fisheries management that focuses on a proxy will then repeatedly believe that recruitment should be higher than it turns out to be. This would in turn lead managers to allow more fishing than the stock can sustain and over time could drive the stock to low levels with imminent risk of collapse. In our model, this is most likely to happen if a number of spawners – recruitment relationship is used, as is commonly done for anadromous fish that are counted as they return from sea to spawn in rivers or freshwater.

Other causes for changing steepness

It should be noted that several mechanisms not included in this model might influence the steepness of a stock–recruitment relationship. For example, in migrating species, there is often an advantage of being large because of the way that swimming costs scale with size (Roff 1988). If life history evolution causes maturation at smaller size, these fish may spend more of their energy budget on the migration and have less for the production of gonads, or they might migrate shorter distances to areas that are less favorable (modeled in Jørgensen et al. 2008). Both of these mechanisms could lower the steepness of a spawning stock biomass – recruitment relationship.

When maturity ogives are outdated

Survey data are always incomplete. In particular, it is often difficult or costly to sample individual maturity status, and spawning stock biomass may be calculated based on a maturity ogive that has been obtained earlier. The ogives used in practical fisheries management are sometimes several years or decades old. For example, the International Council for the Exploration of the Sea (ICES) used a maturity ogive from the early 1980s in their 2009 stock assessment for North Sea plaice (ICES 1983, 2009).

When fishing-induced life history evolution causes earlier maturation, the consequence of using outdated maturity ogives is that the mature part of the stock is underestimated. When the underestimated spawning stock biomass obtained in this way is plotted against recruitment, the stock–recruitment relationship will seem steeper over time. In other words, true recruitment will consistently lie above the expected level predicted from the stock assessment. This likely will lead to a conservative fishing regime in which quotas are set below what fisheries managers would have chosen if they had had a correct view of the spawning stock biomass, and from a sustainability perspective, this is of little concern.

Complications might arise when one begins to look for explanations for the unexpectedly high recruitment. Because evolutionary change is a gradual change over time, any consistent temporal trend may lead to spurious correlations and falsely be identified as the cause for the high recruitment.

Sensitivity analyses

We tested the robustness of the model's predictions by varying two central processes: the strength of density-dependent regulation of individual growth rate and the shape of the total egg production – recruitment relationship (the sensitivity analysis is reported in Supplemental Figs. S1–S4[6]). The qualitative prediction that the change in steepness depends on the stock proxy that is used was not challenged by variation in these processes. For all parameter combinations that we tested, steepness was relatively unchanged when spawning stock biomass was used as proxy, steepness increased with total biomass as proxy, and steepness declined when the proxy was number of spawners.

All species are different, and we cannot claim that these results are general. However, it seems plausible that the model's qualitative predictions form a first expectation for species in which fishing-induced life history evolution leads to earlier maturation. A tendency to mature at younger ages and smaller sizes has been documented in a wide range of species worldwide (listed by Dieckmann et al. 2009), but there are exceptions that can be explained by the intensity and selectivity of the fishery (e.g., Engelhard and Heino 2004).

Implications for management

Fishing-induced evolution is likely to affect life history traits with implications for population-level characteristics and recruitment. Such changes may alter the stock–recruitment relationship, which is often central to stock assessment and management advice.

If the true spawning stock biomass is known, the stock–recruitment relationship changes little due to fishing-induced evolution and there is little concern for fisheries management. Monitoring of maturity status is often costly and time-consuming, however, and we fear that old or outdated maturity data are frequently used in routine stock assessments, especially for small stocks, for stock of little commercial value, or when species biology makes such monitoring particularly challenging. If maturation is changing in these stocks, increased monitoring of maturity status could improve the accuracy of stock assessments by explaining temporal trends in the stock–recruitment relationship.

When management is based on a total stock biomass – recruitment relationship, recruitment may be underestimated, which is of little concern from a sustainability perspective. The most worrisome case is the one in which the number of spawners – recruitment relationship is used to forecast recruitment and to set harvest quotas. For such stocks, current management practice may overestimate recruitment and therefore recommend catches that exceed safe biological limits.

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ERRATUM / ERRATUM

Erratum: Simulating spatial dynamics to evaluate methods of deriving abundance indices for tropical tunas

Thomas R. Carruthers, Murdoch K. McAllister, and Robert N. M. Ahrens

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As noted in the figure captions for Figs. 8 and 9, the third row in each figure (panels *g*, *h*, *i*) illustrates the results in which the relative abundance index is calculated correctly and imputation is applied. However, the third rows of both figures were erroneously labelled in the published article as "Correct calculation, no CPUE imputation". This label should be "Correct calculation, CPUE imputation", as indicated in the figure captions.

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